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L5 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2002:11124 Document No. 136:79766 Inhibitors of platelet activation and recruitment. **Maliszewski, Charles Richard; Gayle, Richard Brownley**; Price, Virginia Lee; Gimpel, Steven Dean (USA). U.S. Pat. Appl. Publ. US 2002002277 A1 20020103, 78 pp., Cont.-in-part of Appl. No. PCT/US99/22955. (English). CODEN: USXXCO. APPLICATION: US 2001-835147 20010413. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813; WO 1999-US22955 19991013.
AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L5 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2000:277996 Document No. 132:303497 **CD39** polypeptides as inhibitors of platelet activation and recruitment. **Maliszewski, Charles R.; Gayle, Richard B., III**; Price, Virginia L.; Gimpel, Steven D. (Immunex Corp., USA). PCT Int. Appl. WO 2000023459 A1 20000427, 122 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US22955 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.
AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L5 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2000:277866 Document No. 132:303495 Methods of inhibiting platelet activation and recruitment. **Maliszewski, Charles R.; Gayle, Richard B., III; Marcus, Aaron J.** (Immunex Corp., USA; Cornell Research Foundation, Inc.). PCT Int. Appl. WO 2000023094 A2 20000427, 118 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM,

CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23641 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

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L6 54 DUP REMOVE L3 (59 DUPLICATES REMOVED)

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L7 0 L6 AND IL-2

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L6 ANSWER 1 OF 54 MEDLINE on STN DUPLICATE 1
2005198163. PubMed ID: 15647328. Ectonucleoside triphosphate diphosphohydrolase 1/**CD39**, localized in neurons of human and porcine heart, modulates ATP-induced norepinephrine exocytosis. Machida Takuji; Heerdt Paul M; Reid Alicia C; Schafer Ulrich; Silver Randi B; Broekman M Johan; **Marcus Aaron J**; Levi Roberto. (Department of Pharmacology, Weill Medical College of Cornell University, New York, NY 10021, USA.) Journal of pharmacology and experimental therapeutics, (2005 May) 313 (2) 570-7. Electronic Publication: 2005-01-12. Journal code: 0376362. ISSN: 0022-3565. Pub. country: United States. Language: English.

AB Using a guinea pig heart synaptosomal preparation, we previously observed that norepinephrine (NE) exocytosis was attenuated by a blockade of P2X purinoceptors, potentiated by inhibition of ectonucleoside triphosphate diphosphohydrolase-1 (E-NTPDase1)/**CD39**, and reduced by soluble **CD39**, a recombinant form of human E-NTPDase1/**CD39**. This suggests that norepinephrine and ATP are coreleased upon depolarization of cardiac sympathetic nerve endings and that ATP enhances norepinephrine exocytosis by an action modulated by E-NTPDase1/**CD39** activity. Whether E-NTPDase1/**CD39** is localized to cardiac neurons and modulates norepinephrine exocytosis in intact heart tissue remained untested. We report that E-NTPDase1/**CD39** is selectively localized in human and porcine cardiac neurons and that depolarization of porcine heart tissue elicits omega-conotoxin-inhibitable release of both norepinephrine and ATP. Inhibition of E-NTPDase1/**CD39** with ARL67156 markedly potentiated ATP release, demonstrating that E-NTPDase1/**CD39** is a major determinant of ATP availability at sympathetic nerve terminals. Notably, inhibition of E-NTPDase1/**CD39** enhanced both ATP and NE exocytosis, whereas administration of soluble **CD39** reduced both ATP and NE exocytosis. The strong correlation between ATP and norepinephrine release was abolished in the presence of the purinergic P2X receptor (P2XR) antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS). We conclude that released ATP governs norepinephrine exocytosis by activating presynaptic P2XR and that this action is controlled by neuronal E-NTPDase1/**CD39**. Clinically, excessive norepinephrine release is a major cause of arrhythmic and coronary vascular dysfunction during myocardial ischemia. By curtailing NE release, in addition to its effects as an antithrombotic agent, soluble **CD39** may constitute a novel therapeutic approach to ischemic complications in the myocardium.

L6 ANSWER 2 OF 54 MEDLINE on STN DUPLICATE 2
2005217609. PubMed ID: 15852226. Role of **CD39** (NTPDase-1) in thromboregulation, cerebroprotection, and cardioprotection. **Marcus Aaron J**; Broekman M Johan; Drosopoulos Joan H F; Olson Kim E; Islam Naziba; Pinsky David J; Levi Roberto. (Weill Medical College of Cornell University, 423 East 23rd Street, New York, NY 10010, USA.. ajmarcus@med.cornell.edu) . Seminars in thrombosis and hemostasis, (2005

Apr) 31 (2) 234-46. Ref: 70. Journal code: 0431155. ISSN: 0094-6176. Pub. country: United States. Language: English.

AB Blood platelets maintain vascular integrity and promote primary and secondary hemostasis following interruption of vessel continuity. Biochemical or physical damage to coronary, carotid, or peripheral arteries promotes excessive platelet activation and recruitment culminating in vascular occlusion and tissue ischemia. Currently, inadequate therapeutic approaches to stroke and coronary artery disease (CAD) are a public health issue. Following our demonstration of neutrophil leukotriene production from arachidonate released from activated aspirin-treated platelets, we studied interactions among platelets and other blood cells. This led to concepts of transcellular metabolism and thromboregulation. Thrombosis has a proinflammatory component whereby biologically active substances are synthesized by different cell types that could not individually synthesize the metabolite(s). Endothelium controls platelet reactivity via at least three biochemical systems: autacoids leading to production of prostacyclin and nitric oxide (NO) and endothelial ecto-adenosine phosphatase (ADPase)/ **CD39**/nucleoside triphosphate diphosphohydrolase (NTPDase-1). The autacoids are fluid phase reactants, not produced by tissues in the basal state, but are only synthesized intracellularly and released upon interactions of cells with an agonist. When released, they exert fleeting actions in the immediate milieu and are rapidly inactivated. **CD39** is an integral component of the endothelial cell (EC) surface and is substrate activated. It maintains vascular fluidity in the complete absence of prostacyclin and NO, indicating that the latter are ancillary components of hemostasis. Therapeutic implications for the autacoids have not been compelling because of their transient and local action and limited potency. Conversely, **CD39**, acting solely on the platelet releasate, is efficacious in animal models. It metabolically neutralizes a prothrombotic releasate via deletion of ADP-the major recruiting agent responsible for formation of an occlusive thrombus. In addition, solCD39 reduced adenosine triphosphate (ATP)- and ischemia-induced norepinephrine release in the heart. This action can prevent fatal arrhythmia. Moreover, solCD39 ameliorated the sequelae of stroke in **cd39** null mice. Thus, **CD39** represents the next generation of cardioprotective and cerebroprotective molecules. This article focuses on our interpretations of recent data and their implications for therapeutics.

L6 ANSWER 3 OF 54 MEDLINE on STN DUPLICATE 3

2005291887. PubMed ID: 15935828. The ratio of ADP- to ATP-ectonucleotidase activity is reduced in patients with coronary artery disease. El-Omar Magdi M; Islam Naziba; Broekman M Johan; Drosopoulos Joan H F; Roa Donald C; Lorin Jeffrey D; Sedlis Steven P; Olson Kim E; Pulte E Dianne; **Marcus Aaron J.** (Department of Medicine-Cardiology, New York University Medical School, New York, NY, USA.) Thrombosis research, (2005) 116 (3) 199-206. Electronic Publication: 2004-12-21. Journal code: 0326377. ISSN: 0049-3848. Pub. country: United States. Language: English.

AB INTRODUCTION: **CD39** (NTPDase1), an endothelial cell membrane glycoprotein, is the predominant ATP diphosphohydrolase (ATPDase) in vascular endothelium. It hydrolyses both triphosphonucleosides and diphosphonucleosides at comparable rates, thus terminating platelet aggregation and recruitment responses to ADP and other platelet agonists. This occurs even when nitric oxide (NO) formation and prostacyclin production are inhibited. Thus, **CD39** represents the main control system for platelet reactivity. Reduced or deficient local ecto-nucleotidase activity may predispose to development of vascular disease. Based on data in animal models and in vitro, **CD39** constitutes a new therapeutic modality for vascular disease with a novel and unique mode of action. MATERIALS AND METHODS: Lymphocytes were isolated from 46 patients with angiographically proven coronary artery disease (CAD) as well as from matched healthy control subjects. Ectonucleotidase ADPase and ATPase activities (prototypical for the ATPDase activity of endothelial cells) were measured using established

radio-TLC procedures. RESULTS AND DISCUSSION: In the patients, a decreased ratio of ADPase to ATPase activities (from 1.26 to 1.04) was observed despite increases in both ADPase and ATPase activities. Coronary artery disease was the only independent predictor of a difference in the ADPase/ATPase activity ratio by multivariate linear regression analysis (P=0.0035). This altered ADPase/ATPase activity ratio in patients may represent a reduction in endogenous defense systems against platelet-driven thrombotic events. These data may identify a population of patients with excessive platelet reactivity in their circulation. Increased generation of prothrombotic ADP in these patients implies a potential benefit from therapeutic intervention with soluble forms of **CD39**.

L6 ANSWER 4 OF 54 MEDLINE on STN DUPLICATE 4

2005393296. PubMed ID: 16052302. Effects of SolCD39, a novel inhibitor of Platelet Aggregation, on Platelet Deposition and Aggregation after PTCA in a Porcine Model. Buergler John M; **Maliszewski Charles R**; Broekman M Johan; Kaluza Grzegorz L; Schulz Daryl G; **Marcus Aaron J**; Raizner Albert E; Kleiman Neal S; Ali Nadir M. (Section of Cardiology, Department of Medicine, Baylor College of Medicine, The Methodist DeBakey Heart Center, Houston, TX 77030, USA.. dwebb@tmh.tmc.edu) . Journal of thrombosis and thrombolysis, (2005 Apr) 19 (2) 115-22. Journal code: 9502018. ISSN: 0929-5305. Pub. country: Netherlands. Language: English.

AB INTRODUCTION: This study evaluated **CD39** in a porcine model of balloon angioplasty and in plasma of patients undergoing percutaneous intervention. **CD39** (E-NTPDase1), is the endothelial ecto-ADPase inhibiting platelet function via hydrolysis of released platelet ADP.methods and results: A recombinant soluble form of **CD39** (solCD39) given intravenously to pigs had an elimination half life of 5--7 days, increased the bleeding time to an extent similar to aspirin, and inhibits platelet aggregation by>90%. Platelet counts and clot retraction remained normal following solCD39 administration. In a pig model of acute coronary balloon injury, solCD39 resulted in non-statistically significant decreases in platelet (7.7+/-1.4 versus 11.7+/- 3.4) and fibrin (3.5+/-0.4 versus 4.2+/- 0.7) deposition ratios. Adding ex vivo to human platelet rich plasma (PRP) solCD39 produced nearly 100% inhibition of ADP-induced platelet aggregation. A dose-response effect of solCD39 on platelet aggregation induced by collagen or a thrombin receptor activating peptide (TRAP(SFLLRN)) was noted in PRP obtained from volunteers and patients receiving aspirin, clopidogrel or ticlopidine. SolCD39 also provided additional and complete inhibition of TRAP-induced platelet aggregation in PRP from patients who had received abciximab, aspirin and clopidogrel.Conclusions: SolCD39, a novel inhibitor of platelet activation and recruitment with a relatively long half-life appears to be well tolerated and is a potent inhibitor of ADP-, collagen-, or TRAP-induced platelet activation. Its potential use in percutaneous coronary intervention requires further study.Abbreviated Abstract. E-NTPDase1/ **CD39** is the endothelial ecto-ADPase responsible for inhibition of platelet function. A recombinant soluble form (solCD39) had an elimination half life of 5-7 days in pigs, elevated bleeding times similar to aspirin, did not affect clot retraction, and inhibited platelet aggregation by > 90%. When combined with standard heparin therapy in a pig model of acute coronary balloon injury, solCD39 resulted in a trend toward a decrease in platelet and fibrin deposition. SolCD39 added ex vivo to human platelet rich plasma yielded nearly 100% inhibition of ADP-induced platelet aggregation and provided further inhibition when combined with standard therapy.

L6 ANSWER 5 OF 54 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2005059886 EMBASE Erratum: Heterologous cell interactions: Thromboregulation, cerebroprotection and cardioprotection by **CD39** (NTPDase-1) (Journal of Thrombosis Haemostasis (2003) vol. 1 (2497-2509)). **Marcus A.J.**; Broekman M.J.; Drosopoulos J.H.F.; Islam N.; Pinsky D.J.; Sesti

C.; Levi R.. Journal of Thrombosis and Haemostasis Vol. 2, No. 4, pp. 682 2004.
ISSN: 1538-7933. CODEN: JTHOA5
Pub. Country: United Kingdom. Language: English.
ED Entered STN: 20050218
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L6 ANSWER 6 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:364078 The Genuine Article (R) Number: 809HL. Heterologous cell-cell interactions: thromboregulation, cerebroprotection and cardioprotection by **CD39** (NTPDase-1) (vol 1, pg 2497, 2003). **Marcus A J** **(Reprint)**; Broekman M J; Drosopoulos J H F; Islam N; Pinsky D J; Sesti C; Levi R. JOURNAL OF THROMBOSIS AND HAEMOSTASIS (APR 2004) Vol. 2, No. 4, pp. 682-682. ISSN: 1538-7933. Publisher: BLACKWELL PUBL LTD, 108 COWLEY RD, OXFORD OX4 1JF, OXON, ENGLAND. Language: English.

L6 ANSWER 7 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:291932 The Genuine Article (R) Number: 871JM. ATP-induced modulation of norepinephrine exocytosis in human and porcine heart: Role of E-NTPDase1/ **CD39**.. Machida T (Reprint); Heerdt P M; Reid A C; Schaefer U; Silver R B; Broekman M J; **Marcus A J**; Levi R. Cornell Univ, Weill Med Coll, New York, NY 10021 USA; Weill Cornell Med Coll, VA NY Harbor Healthcare Syst, New York, NY USA. BLOOD (16 NOV 2004) Vol. 104, No. 11, Part 1, pp. 517A-517A. MA 1869. ISSN: 0006-4971. Publisher: AMER SOC HEMATOLOGY, 1900 M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA. Language: English.

L6 ANSWER 8 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:608484 The Genuine Article (R) Number: 866OT. Deletion of endothelial ectoapyrase (**CD39**) promotes atherogenesis in hyperlipidemic mice . Mazer S P (Reprint); Fedarau M; Liu Y L; Hwang D W; Towe C W; Liu C F; Olson K E; Broekman M J; **Marcus A J**; Deisher T A; Pinsky D J. Columbia Univ, New York, NY USA; Cornell Univ, Weill Med Coll, New York, NY USA; Amgen Corp, Thousand Oaks, CA USA; Univ Michigan, Ann Arbor, MI 48109 USA. CIRCULATION (26 OCT 2004) Vol. 110, No. 17, Supp. [S], pp. 79-79. MA 372. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 9 OF 54 MEDLINE on STN DUPLICATE 5
2003594985. PubMed ID: 14675084. Heterologous cell-cell interactions: thromboregulation, cerebroprotection and cardioprotection by **CD39** (NTPDase-1). **Marcus A J**; Broekman M J; Drosopoulos J H F; Islam N; Pinsky D J; Sesti C; Levi R. (Department of Medicine, Weill Medical College of Cornell University, and Medical Service/Hematology-Oncology, VA New York Harbor Healthcare System, New York, NY 10010, USA.. ajmarcus@med.cornell.edu) . Journal of thrombosis and haemostasis : JTH, (2003 Dec) 1 (12) 2497-509. Ref: 59. Journal code: 101170508. ISSN: 1538-7933. Pub. country: England: United Kingdom. Language: English.
AB Blood platelets maintain vascular integrity and promote primary and secondary hemostasis following interruption of vessel continuity. Biochemical or physical damage to the coronary, carotid or peripheral arteries is followed by excessive platelet activation and recruitment culminating in vascular occlusion and tissue ischemia. Currently inadequate therapeutic approaches to stroke and coronary artery disease are a public health issue. Following our demonstration of neutrophil leukotriene production from arachidonate released from activated aspirin-treated platelets, we studied interactions between platelets and other blood cells, leading to concepts of transcellular metabolism and thromboregulation. Thrombosis has a proinflammatory component whereby biologically active substances are synthesized by interactions between different cell types that could not individually synthesize the product(s). Endothelial cells control platelet reactivity via three

biochemical systems-autacoids leading to production of prostacyclin and nitric oxide, and endothelial ecto-ADPase/**CD39**/NTPDase-1. The autacoids are fluid-phase reactants, not produced by tissues in the basal state. They are only synthesized intracellularly and released upon interactions of cells with an agonist. When released, autacoids exert fleeting actions in the immediate milieu, and are rapidly inactivated. **CD39** is an integral component of the endothelial cell surface and is substrate-activated. It maintains vascular fluidity in the complete absence of prostacyclin and nitric oxide, indicating that they are ancillary components of hemostasis. Therapeutic implications for the autacoids have not been compelling because of their transient, local and fleeting action, and limited potency. Conversely, **CD39**, acting solely on the platelet releasate, is efficacious in three different animal models. It metabolically neutralizes a prothrombotic platelet releasate via deletion of ADP--the major recruiting agent responsible for formation of an occlusive thrombus. In addition, solCD39 reduced ATP- and ischemia-induced norepinephrine release in the heart. This reduction can prevent fatal arrhythmia. Moreover, solCD39 ameliorated the sequelae of stroke in **CD39** null mice. **CD39** represents the next generation of cardioprotective and cerebroprotective molecules.

L6 ANSWER 10 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:535805 The Genuine Article (R) Number: 691NW. Ectonucleotidase in sympathetic nerve endings modulates ATP and norepinephrine exocytosis in myocardial ischemia. Sesti C; Koyama M; Broekman M J; **Marcus A J** ; Levi R (Reprint). Cornell Univ, Weill Med Coll, Dept Pharmacol, Room LC419, 1300 York Ave, New York, NY 10021 USA (Reprint); VA New York Harbor Hlth Care Syst, Dept Pharmacol, New York, NY USA; VA New York Harbor Hlth Care Syst, Dept Med, Div Hematol & Med Oncol, New York, NY USA; Cornell Univ, Weill Med Coll, Dept Med, Div Hematol & Med Oncol, New York, NY USA; Cornell Univ, Weill Med Coll, Dept Pathol, New York, NY USA. JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS (JUL 2003) Vol. 306, No. 1, pp. 238-244. ISSN: 0022-3565. Publisher: AMER SOC PHARMACOLOGY EXPERIMENTAL THERAPEUTICS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We recently reported that ATP, coreleased with norepinephrine (NE) from cardiac sympathetic nerves, increases NE exocytosis via a positive feedback mechanism. A neuronal ectonucleotidase (E-NTPDase) metabolizes the released ATP, decreasing NE exocytosis. Excessive NE release in myocardial ischemia exacerbates cardiac dysfunction. Thus, we studied whether the ATP-mediated autocrine amplification of NE release is operative in ischemia and, if so, whether it can be modulated by E-NTPDase and its recombinant equivalent, solCD39. Isolated, guinea pig hearts underwent 10- or 20-min ischemic episodes, wherein NE was released by exocytosis and reversal of the NE transporter, respectively. Furthermore, to restrict the role of E-NTPDase to transmitter ATP, sympathetic nerve endings were isolated (cardiac synaptosomes) and subjected to increasing periods of ischemia. Availability of released ATP at the nerve terminals was either increased via E-NTPDase inhibition or diminished by enhancing ATP hydrolysis with solCD39. P2X receptor blockade with PPADS was used to attenuate the effects of released ATP. We found that, in short-term ischemia (but, as anticipated, not in protracted ischemia, where NE release is carrier-mediated), ATP exocytosis was linearly correlated with that of NE. This indicates that by limiting the availability of ATP at sympathetic terminals, E-NTPDase effectively attenuates NE exocytosis in myocardial ischemia. Our findings suggest a key role for neuronal E-NTPDase in the control of adrenergic function in the ischemic heart. Because excessive NE release is an established cause of dysfunction in ischemic heart disease, solCD39 may offer a novel therapeutic approach to myocardial ischemia and its consequences.

L6 ANSWER 11 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2004:95485 The Genuine Article (R) Number: 739RQ. Downregulation of endothelial **CD39** expression by hypoxic or ischemic stress. Yoshikawa Y (Reprint); Mazer S P; Olson K E; Hui L; Broekman M J; **Marcus A J**; Pinsky D J. Columbia Univ, New York, NY USA; Weill Cornell Med Coll, New York, NY USA; VA NY Healthcare Syst, New York, NY USA; Univ Michigan, Ann Arbor, MI 48109 USA. CIRCULATION (28 OCT 2003) Vol. 108, No. 17, Supp. [S], pp. 111-111. MA 522. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 12 OF 54 MEDLINE on STN DUPLICATE 6
2003134850. PubMed ID: 12649347. Metabolic control of excessive extracellular nucleotide accumulation by **CD39** /ecto-nucleotidase-1: implications for ischemic vascular diseases. **Marcus Aaron J**; Broekman M Johan; Drosopoulos Joan H F; Islam Naziba; Pinsky David J; Sesti Casilde; Levi Roberto. (Department of Medicine, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10010, USA.. ajmarcus@med.cornell.edu) . Journal of pharmacology and experimental therapeutics, (2003 Apr) 305 (1) 9-16. Ref: 43. Journal code: 0376362. ISSN: 0022-3565. Pub. country: United States. Language: English.

AB Platelets are responsible for maintaining vascular integrity. In thrombocytopenic states, vascular permeability and fragility increase, presumably due to the absence of this platelet function. Chemical or physical injury to a blood vessel induces platelet activation and platelet recruitment. This is beneficial for the arrest of bleeding (hemostasis), but when an atherosclerotic plaque is ulcerated or fissured, it becomes an agonist for vascular occlusion (thrombosis). Experiments in the late 1980s cumulatively indicated that endothelial cell **CD39**-an ecto-ADPase-reduced platelet reactivity to most agonists, even in the absence of prostacyclin or nitric oxide. As discussed herein, **CD39** rapidly and preferentially metabolizes ATP and ADP released from activated platelets to AMP, thereby drastically reducing or even abolishing platelet aggregation and recruitment. Since ADP is the final common agonist for platelet recruitment and thrombus formation, this finding highlights the significance of **CD39**. A recombinant, soluble form of human **CD39**, solCD39, has enzymatic and biological properties identical to the full-length form of the molecule and strongly inhibits human platelet aggregation induced by ADP, collagen, arachidonate, or TRAP (thrombin receptor agonist peptide). In sympathetic nerve endings isolated from guinea pig hearts, where neuronal ATP enhances norepinephrine exocytosis, solCD39 markedly attenuated norepinephrine release. This suggests that NTPDase (nucleoside triphosphate diphosphohydrolase) could exert a cardioprotective action by reducing ATP-mediated norepinephrine release, thereby offering a novel therapeutic approach to myocardial ischemia and its consequences. In a murine model of stroke, driven by excessive platelet recruitment, solCD39 reduced the sequelae of stroke, without an increase in intracerebral hemorrhage. **CD39** null mice, generated by deletion of apyrase-conserved regions 2 to 4, exhibited a decrease in postischemic perfusion and an increase in cerebral infarct volume when compared with controls. "Reconstitution" of **CD39** null mice with solCD39 reversed these changes. We hypothesize that solCD39 has potential as a novel therapeutic agent for thrombotic diatheses.

L6 ANSWER 13 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:62767 Document No.: PREV200400063168. Downregulation of endothelial **CD39** expression by hypoxic or ischemic stress. Yoshikawa, Yasushi [Reprint Author]; Mazer, Sean P. [Reprint Author]; Olson, Kim E. [Reprint Author]; Liao, Hui [Reprint Author]; Broekman, M. Johan; **Marcus, Aaron J.**; Pinsky, David J.. Columbia Univ, New York, NY, USA. Circulation, (October 28 2003) Vol. 108, No. 17 Supplement, pp. IV-111. print.
Meeting Info.: American Heart Association Scientific Sessions 2003.

Orlando, FL, USA. November 09-12, 2003. American Heart Association. ISSN: 0009-7322 (ISSN print). Language: English.

L6 ANSWER 14 OF 54 CAPLUS COPYRIGHT 2005 ACS on STN
2002:11124 Document No. 136:79766 Inhibitors of platelet activation and recruitment. **Maliszewski, Charles Richard; Gayle, Richard Brownley**; Price, Virginia Lee; Gimpel, Steven Dean (USA). U.S. Pat. Appl. Publ. US 2002002277 A1 20020103, 78 pp., Cont.-in-part of Appl. No. PCT/US99/22955. (English). CODEN: USXXCO. APPLICATION: US 2001-835147 20010413. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813; WO 1999-US22955 19991013.
AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L6 ANSWER 15 OF 54 MEDLINE on STN DUPLICATE 7
2002230961. PubMed ID: 11956240. Elucidation of the thromboregulatory role of **CD39**/ectoapyrase in the ischemic brain. Pinsky David J; Broekman M Johan; Peschon Jacques J; Stocking Kim L; Fujita Tomoyuki; Ramasamy Ravichandran; Connolly E Sander Jr; Huang Judy; Kiss Szilard; Zhang Yuan; Choudhri Tanvir F; McTaggart Ryan A; Liao Hui; Drosopoulos Joan H F; Price Virginia L; **Marcus Aaron J**; **Maliszewski Charles R.** (Division of Cardiology, Department of Medicine, College of Physicians and Surgeons, Columbia University, Presbyterial Hospital 10 Stem, 630 W 168th Street, New York, NY 10032, USA.. djp5@columbia.edu) . Journal of clinical investigation, (2002 Apr) 109 (8) 1031-40. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB Endothelial **CD39** metabolizes ADP released from activated platelets. Recombinant soluble human **CD39** (solCD39) potently inhibited ex vivo platelet aggregation in response to ADP and reduced cerebral infarct volumes in mice following transient middle cerebral artery occlusion, even when given 3 hours after stroke. Postischemic platelet and fibrin deposition were decreased and perfusion increased without increasing intracerebral hemorrhage. In contrast, aspirin did not increase postischemic blood flow or reduce infarction volume, but did increase intracerebral hemorrhage. Mice lacking the enzymatically active extracellular portion of the **CD39** molecule were generated by replacement of exons 4-6 (apyrase-conserved regions 2-4) with a PGKneo cassette. Although **CD39** mRNA 3' of the neomycin cassette insertion site was detected, brains from these mice lacked both apyrase activity and **CD39** immunoreactivity. Although their baseline phenotype, hematological profiles, and bleeding times were normal, **cd39**(-/-) mice exhibited increased cerebral infarct volumes and reduced postischemic perfusion. solCD39 reconstituted these mice, restoring postischemic cerebral perfusion and rescuing them from cerebral injury. These data demonstrate that **CD39** exerts a protective thromboregulatory function in stroke.

L6 ANSWER 16 OF 54 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 8
2002338303 EMBASE COX inhibitors and thromboregulation. **Marcus A.J.** ; Broekman M.J.; Pinsky D.J.. Dr. A.J. Marcus, Vet. Aff. NY Harbor Healthcare Syst., New York, NY 10010, United States. New England Journal of Medicine Vol. 347, No. 13, pp. 1025-1026 26 Sep 2002.
Refs: 5.
ISSN: 0028-4793. CODEN: NEJMAG
Pub. Country: United States. Language: English.
ED Entered STN: 20021017
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L6 ANSWER 17 OF 54 MEDLINE on STN DUPLICATE 9
2002499227. PubMed ID: 12271274. Role of extracellular ATP metabolism in regulation of platelet reactivity. Birk Alex V; Broekman M Johan; Gladek Eva M; Robertson Hugh D; Drosopoulos Joan H F; **Marcus Aaron J**;

Szeto Hazel H. (Department of Pharmacology, Weill Medical College of Cornell University, New York, NY 10021, USA.) Journal of laboratory and clinical medicine, (2002 Sep) 140 (3) 166-75. Journal code: 0375375. ISSN: 0022-2143. Pub. country: United States. Language: English.

AB Extracellular adenosine triphosphate (ATP) regulates platelet reactivity by way of direct action on platelet purinergic receptors or by hydrolysis to adenosine diphosphate (ADP). Subsequent metabolism of ATP and ADP to adenosine monophosphate (AMP) and adenosine inhibits platelet aggregation. Endothelial cell membrane-bound ecto-ATP/ADPase (**CD39**, E-NTPDase1) is thought to be the main regulator of platelet responsiveness. However, the findings in studies of **CD39** -knockout mice imply that nucleotidase(s) in plasma regulates circulating adenine nucleotides levels. Understanding extracellular ATP metabolism by **CD39** and plasma nucleotidases is therefore important. In this study, alpha-phosphorus 32- and gamma-phosphorus 32-labeled ATP were rapidly metabolized directly to AMP and pyrophosphate in human plasma at pH 7.4, suggesting the presence of pyrophosphatase/phosphodiesterase-like activity. A specific phosphodiesterase substrate, p-nitrophenol-5'-TMP (p-Nph-5'-TMP), was readily hydrolyzed in human plasma. The antiaggregatory action of beta,gamma-methylene-ATP (AMPPCP) (5 micromol/L) was blocked by DMPX, an adenosine-receptor antagonist, suggesting that in plasma, AMPPCP was metabolized to AMP and adenosine. Recombinant soluble **CD39** (solCD39) was used to assess the role of **CD39** in ATP metabolism. As little as 0.25 microg/mL of solCD39 inhibited ADP-induced platelet aggregation. However, in the presence of ADP-free ATP (10 micromol/L), solCD39 induced platelet aggregation in a dose-dependent manner. Because AMPPCP could not substitute for ATP in solCD39-stimulated platelet aggregation, it is likely that ADP formation from ATP was required. Endogenous **CD39** may thus have a hemostatic function by promoting ADP formation from released ATP, in addition to its antiaggregatory properties. A plasma nucleotidase hydrolyzes ATP directly to AMP. This prevents ADP accumulation and generates adenosine, a potent, locally acting inhibitor of platelet reactivity. The presence of both endothelial **CD39** and plasma nucleotidase appears to be important in the maintenance of normal hemostasis and prevention of excessive platelet responsiveness.

L6 ANSWER 18 OF 54 MEDLINE on STN DUPLICATE 10

2002192153. PubMed ID: 11919550. Role of a novel soluble nucleotide phospho-hydrolase from sheep plasma in inhibition of platelet reactivity: hemostasis, thrombosis, and vascular biology. Birk Alex V; Bubman Darya; Broekman M Johan; Robertson Hugh D; Drosopoulos Joan H F; **Marcus Aaron J**; Szeto Hazel H. (Division of Hematology and Medical Oncology, Department of Medicine, Weill Medical College of Cornell University, New York, New York 10021, USA.) Journal of laboratory and clinical medicine, (2002 Feb) 139 (2) 116-24. Journal code: 0375375. ISSN: 0022-2143. Pub. country: United States. Language: English.

AB Ecto- and exoenzymes that metabolize extracellular adenosine diphosphate (ADP), the major promoter of platelet activation and recruitment, are of potential clinical importance because they can metabolically prevent excessive thrombus growth. An ecto-ADPase (**CD39**, NTPDase1) has been identified on endothelial cells. We demonstrate that ADP and adenosine triphosphate (ATP) are rapidly metabolized to adenosine monophosphate (AMP) in sheep plasma at pH 7.4. This hydrolysis is sensitive to P(1), P(5)-di-(adenosine-5') pentaphosphate (Ap(5)A), and ethylene glycol bis (beta-aminoethyl ether) - N, N, N(-), N(-) tetra-acetate (EGTA) but insensitive to tetramisole (an alkaline phosphatase inhibitor). A specific phosphodiesterase substrate, p -nitrophenol-5'-thymidine monophosphate (TMP) (p -Nph-5'-TMP), was readily hydrolyzed in sheep plasma at a rate of approximately 0.25 nmol/min/mg protein, and this hydrolysis was inhibited by ADP, ATP, and Ap(5)A. Furthermore, 200-fold purified p -Nph-5'-TMP-hydrolyzing activity also hydrolyzed ATP and ADP directly to AMP. When ADP was preincubated in plasma, its ability to induce platelet aggregation was inhibited in a time-dependent manner. This effect was abolished by Ap(5)A. The

inhibitory effects on platelet aggregation correlated with hydrolysis of the ADP in plasma. These data suggest that the endogenous soluble plasma phosphohydrolase metabolizes ATP and ADP by means of cleavage of the alpha-beta-phosphodiester bond of nucleoside 5'-phosphate derivatives. This novel biochemical activity inhibits platelet reactivity through hydrolysis of extracellular nucleotides released by activated platelets during (patho)physiological processes, serving a homeostatic and antithrombotic function in vivo.

L6 ANSWER 19 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:336630 Document No.: PREV200300336630. ATP/ADP Ectonucleotidase Activity Is Increased in Patients with Coronary Artery Disease. El-Omar, Magdi M. [Reprint Author]; Islam, Naziba; Broekman, M. Johan; Drosopoulos, Joan H. F.; Roa, Donald C.; Lorin, Jeffrey; Sedlis, Steven P.; **Marcus, Aaron J.**. Medicine, VA New York Harbor Healthcare System, New York, NY, USA. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 1932. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB **CD39** (E-NTPDase-1) is the predominant ectonucleotidase at the luminal surface of blood vessels as well as on lymphocytes. Recent evidence from our group indicates that **CD39** plays a key role in thromboregulation by hydrolyzing ADP released from activated platelets, while also metabolizing ATP at a similar rate. In a murine model of stroke, endogenous and/or exogenously administered **CD39** limited the sequelae of stroke. The higher the ratio of ATPase/ADPase activity of an ectonucleotidase, the lower its platelet anti-aggregatory effect. In the present study we examined ectonucleotidase ATPase and ADPase activities in patients with coronary artery disease (CAD). Males with angiographically-documented CAD (gtoreq1 major vessel with gtoreq50% stenosis) were compared to a group of age-matched healthy males without apparent CAD (controls). Lymphocytes were isolated from heparinized whole blood using Histopaque gradient centrifugation, and washed prior to incubation with 50muM 14C ADP or 14C ATP (5 min, 37degreeC). Thin layer chromatography (TLC) was used to separate nucleotides, nucleosides and bases, and radioactivity was quantified by radio-TLC scanning. Data were expressed as pmoles ATP or ADP metabolized per min per 5x104 lymphocytes, and ATPase/ADPase activity ratios were calculated. Results: ATPase activity was higher in CAD compared to controls, despite similar ADPase activity. The ratio of ATPase/ADPase activity was significantly higher (apprx20%, $p<0.005$) in CAD compared to controls (Table). Conclusion: Patients with CAD have an increased ATP/ADP ectonucleotidase activity ratio, possibly due to an alteration in **CD39** nucleotide specificity and/or co-expression of another ectonucleotidase with greater ATP specificity. The altered ATPase/ADPase activity ratio may lower endogenous defenses against platelet-driven thrombotic events in these patients. The results suggest that NTPDase-1 or recombinant derivatives therefrom may represent a novel therapeutic modality to occlusive vascular diseases.

L6 ANSWER 20 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:395879 Document No.: PREV200100395879. Thromboregulation by endothelial cells: Significance for occlusive vascular diseases: Response. **Marcus, Aaron J.** [Reprint author]; Broekman, M. Johan [Reprint author]; Drosopoulos, Joan H. F. [Reprint author]. Hematology-Oncology, VA-New York Harbor Healthcare System/Weill Medical College of Cornell University, New York, NY, USA. Arteriosclerosis Thrombosis and Vascular Biology, (July, 2001) Vol. 21, No. 7, pp. 1251-1252. print.
ISSN: 1079-5642. Language: English.

L6 ANSWER 21 OF 54 EMBASE

on STN DUPLICATE 11
2002098539 EMBASE Thromboregulation by endothelial cells: Significance for occlusive vascular diseases (multiple letters). Robson S.C.; **Marcus A.J.**; Broekman M.J.; Drosopoulos J.H.F.. Prof. S.C. Robson, Department of Medicine, Harvard Medical School Boston, Boston, MA, United States. Arteriosclerosis, Thrombosis, and Vascular Biology Vol. 21, No. 7, pp. 1251-1252 2001.
ISSN: 1079-5642. CODEN: ATVBFA
Pub. Country: United States. Language: English.
ED Entered STN: 20020328
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L6 ANSWER 22 OF 54 MEDLINE on STN DUPLICATE 12
2002043730. PubMed ID: 11770867. Inhibition of platelet recruitment by endothelial cell **CD39**/ecto-ADPase: significance for occlusive vascular diseases. **Marcus A J**; Broekman M J; Drosopoulos J H; Pinsky D J; Islam N; Maliszewsk C R. (Department of Medicine, VA New York Harbor Healthcare System and Weill Medical College of Cornell University, NY 10010, USA.. ajmarcus@med.cornell.edu) . Italian heart journal : official journal of the Italian Federation of Cardiology, (2001 Nov) 2 (11) 824-30. Journal code: 100909716. ISSN: 1129-471X. Pub. country: Italy. Language: English.
AB During their 7-9 day lifespan in the circulation platelets are mainly responsible for maintaining the integrity of the vasculature. In thrombocytopenic states, there is an increase in vascular permeability and fragility, presumably due to absence of this platelet function. In sharp contrast, biochemical or physical injury in the coronary, carotid or peripheral arteries induces platelet activation and platelet recruitment, which can culminate in thrombotic vascular occlusion. Since there is one death every 33 s from vascular occlusion in the United States, this situation constitutes a major public health issue. In the course of studying interactions between cells of the vascular wall and those in the circulation, we observed that platelets in close proximity to endothelial cells do not respond to agonists in vitro. Experiments initiated in the late 1980's cumulatively indicated that endothelial cell **CD39** --an ecto-ADPase--was mainly responsible for this phenomenon. **CD39** rapidly and preferentially metabolizes ADP released from activated platelets. ADP is the final common pathway for platelet recruitment and thrombus formation, and platelet aggregation and recruitment are abolished by **CD39**. Our current hypothesis is that **CD39** will be a novel antithrombotic agent for treating high risk patients who have activated platelets in their circulation--the identifying characteristic of coronary artery occlusion and thrombotic stroke. A recombinant, soluble form of human **CD39** has been generated. This is solCD39, a glycosylated protein of 66 kDa whose enzymatic and biological properties are identical to the full-length form of the enzyme. In our in vitro experiments, solCD39 blocks ADP-induced human platelet aggregation, and inhibits collagen- and thrombin receptor agonist peptide-induced platelet reactivity. We studied solCD39 in vitro in a murine model of stroke, which was shown to be driven by excessive platelet recruitment. In studies with **CD39** wild-type (**CD39**+/+) mice solCD39 completely abolished ADP-induced platelet aggregation, and strongly inhibited collagen- and arachidonate-induced platelet reactivity ex vivo. When solCD39 was administered prior to transient intraluminal middle cerebral artery occlusion, it reduced ipsilateral fibrin deposition, decreased (111)In-platelet deposition, and increased post-ischemic blood flow 2-fold at 24 hours. These results were superior to those we obtained with aspirin pre-treatment. **CD39** null (**CD39**-/-) mice, which we generated by deletion of exons 4-6 (apyrase conserved regions 2-4), have a normal phenotype, normal hematologic profiles and bleeding times, but exhibit a decrease in post-ischemic perfusion and an increase in cerebral infarct volume when compared to genotypic **CD39**+/+ controls in our stroke model. "Reconstitution" of **CD39** null mice with solCD39 reversed these pathologic changes. Thus, the **CD39**-/- mice were actually

rescued from cerebral injury by solCD39, thereby fulfilling Koch's postulates. These experiments have led us to hypothesize that solCD39 has potential as a novel therapeutic agent for thrombotic stroke. In this review, we summarize our recent research results with **CD39** and solCD39, and discuss our viewpoints on its present and future possibilities as a novel treatment for thrombosis.

L6 ANSWER 23 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:214774 The Genuine Article (R) Number: 389JF. Soluble **CD39** but not aspirin decreases platelet deposition and improves outcome in reperfused murine stroke. Kiss S (Reprint); **Marcus A J**; Broekman M J; Nair M N; D'Ambrosio A L; Liao H; **Maliszewski C R**; Connolly E S; Pinsky D J. Columbia Univ Coll Phys & Surg, New York, NY 10032 USA; Cornell Univ, Weill Med Coll, New York, NY USA; Immunex Res & Dev Corp, Seattle, WA 98101 USA. STROKE (JAN 2001) Vol. 32, No. 1, pp. 359-359. MA P109. ISSN: 0039-2499. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 24 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:936710 The Genuine Article (R) Number: 487UW. Leukoregulatory role of **CD39** in ischemic vessels. Fujita T (Reprint); **Maliszewski C R**; Liao H; Okada K; **Marcus A J**; Broekman M J; Ramasamy R; Pinsky D J. Columbia Univ, New York, NY USA; Immunex Corp, Seattle, WA USA; Columbia Univ, Weill Med Coll, New York, NY USA. CIRCULATION (23 OCT 2001) Vol. 104, No. 17, Supp. [S], pp. 274-274. MA 1316. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 25 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:198682 Document No.: PREV200200198682. Protective effect of ecto-nucleotidases: Modulation of ATP-induced norepinephrine release during myocardial ischemia. Levi, Roberto [Reprint author]; Koyama, Motohiro [Reprint author]; Sesti, Casilde [Reprint author]; Broekman, Marinus J.; Drosopoulos, Joan H. F.; Islam, Naziba; **Marcus, Aaron J.**. Pharmacology, Weill Cornell Medical College, New York, NY, USA. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 248a. print. Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Ecto-nucleotidases are homeostatic enzymes that serve a protective role in thromboregulation and purinergic signaling. Thus, endothelial cell E-NTPDase1/**CD39** metabolically deletes nucleotides (ATP and ADP) released from activated platelets, thereby inhibiting recruitment and thrombus formation. We recently reported that ATP, co-released with norepinephrine (NE) from cardiac sympathetic nerve terminals (synaptosomes), increases NE release via a positive feedback mechanism. During ischemia additional ATP is secreted, amplifying further NE release, causing serious arrhythmias. To assess control mechanisms of ATP release, we determined that cardiac synaptosomes do indeed express ecto-nucleotidase activities (calcium-dependent ecto-ATPase and ecto-ADPase), as measured by metabolism of exogenously added radiolabeled ATP or ADP. Furthermore, classical E-NTPDase inhibitors such as ARL67156 (ARL), diethylpyrocarbonate (DEPC), and 10 mM azide attenuated these activities. Inhibitors of alkaline phosphatase (tetramisole), adenylate kinase (Ap5A), or ATP-dependent pump enzymes (ouabain, 1 mM azide) had no effect. Cardiac ecto-nucleotidase activities were not enhanced by concanavalin A, in contrast to its effects on human E-NTPDase1, and its soluble derivative, solCD39. Synaptosomal ecto-nucleotidase activity was 1.7-fold greater for ATP than for ADP. SolCD39 as a prototypical ENTPDase1 had a 1.3-fold preference for ADP in parallel experiments.

Thus, cardiac synaptosomes express an ecto-nucleotidase resembling ENTPDase1. Little or no adenosine was generated in this system, indicating the absence of 5'-nucleotidase activity. In isolated guinea-pig hearts undergoing ischemia-reperfusion, inhibition of ecto-nucleotidase activity with ARL markedly increased NE release (50% over untreated ischemic controls). Conversely, administration of solCD39 reduced NE release by 50%. Moreover, synaptosomal NE release, elicited by exogenous ATP, was potentiated by ARL, and abrogated by solCD39. Similarly, ARL potentiated the synaptosomal NE release elicited by K+-induced depolarization, demonstrating that release of NE is amplified by co-released ATP. Collectively our data demonstrate that cardiac sympathetic nerve terminals possess ecto-nucleotidase activities that modulate excessive NE release in normal and pathophysiological conditions such as myocardial ischemia. By controlling neurotransmitter release, these synaptosomal nucleotidase(s) exert a cardioprotective function. Our findings suggest that solCD39, in addition to its antithrombotic effects in coronary occlusion and stroke, may also be useful in therapeusis for myocardial ischemia by reducing the morbidity of NE-induced arrhythmias.

L6 ANSWER 26 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 13

2001:251364 The Genuine Article (R) Number: 412PH. Thromboregulation by endothelial cells - Significance for occlusive vascular diseases. **Marcus A J (Reprint)**; Broekman M J; Drosopoulos J H F; Pinsky D J; Islam N; **Gayle R B**; **Maliszewski C R.** VA New York Harbor Healthcare Syst, 423 E 23rd St, Room 13028W, New York, NY 10010 USA (Reprint); VA New York Harbor Healthcare Syst, New York, NY 10010 USA; Cornell Univ, Weill Med Coll, New York, NY USA; Columbia Univ Coll Phys & Surg, New York, NY 10032 USA; Immunex Corp, Seattle, WA USA. ARTERIOSCLERO SIS THROMBOSIS AND VASCULAR BIOLOGY (FEB 2001) Vol. 21, No. 2, pp. 178-182 . ISSN: 1079-5642. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB During their 7- to 9-day lifespan in the circulation, platelets perform an ill-defined baseline function that maintains the integrity of the vasculature. In thrombocytopenic states, there is an increase in vascular permeability and fragility, which is presumably due to absence of this platelet function. In sharp contrast, biochemical or physical injury in the coronary, carotid, or peripheral arteries induces platelet activation and platelet recruitment, which can progress to thrombotic vascular occlusion. Because there is 1 death every 33 seconds from vascular occlusion in the United States, this problem has critical public health implications. In this review, we describe the characterization of a novel potential antithrombotic agent with a unique mode of action--biochemical "deletion" of ADP from an activated platelet releasate, which thereby inhibits platelet recruitment and further activation.

L6 ANSWER 27 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:274993 Document No.: PREV200200274993. Leukoregulatory role of **CD39** in ischemic vessels. Fujita, Tomoyuki [Reprint author]; **Maliszewski, Charles R.**; Liao, Hui; Okada, Kenji; **Marcus, Aaron J.**; Broekman, M. Johann; Ramasamy, Ravichandran; Pinsky, David J.. Columbia Univ, New York, NY, USA. Circulation, (October 23, 2001) Vol. 104, No. 17 Supplement, pp. II.274. print.
Meeting Info.: Scientific Sessions 2001 of the American Heart Association. Anaheim, California, USA. November 11-14, 2001. American Heart Association.
CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

L6 ANSWER 28 OF 54 CAPLUS COPYRIGHT 2005 ACS on STN

2000:277996 Document No. 132:303497 **CD39** polypeptides as inhibitors of platelet activation and recruitment. **Maliszewski, Charles R.**; **Gayle, Richard B., III**; Price, Virginia L.;

Gimpel, Steven D. (Immunex Corp., USA). PCT Int. Appl. WO 2000023459 A1 20000427, 122 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US22955 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L6 ANSWER 29 OF 54 CAPLUS COPYRIGHT 2005 ACS on STN

2000:277866 Document No. 132:303495 Methods of inhibiting platelet activation and recruitment. **Maliszewski, Charles R.; Gayle, Richard B., III; Marcus, Aaron J.** (Immunex Corp., USA; Cornell Research Foundation, Inc.). PCT Int. Appl. WO 2000023094 A2 20000427, 118 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23641 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L6 ANSWER 30 OF 54 MEDLINE on STN DUPLICATE 14

2000302517. PubMed ID: 10841775. Site-directed mutagenesis of human endothelial cell ecto-ADPase/soluble **CD39**: requirement of glutamate 174 and serine 218 for enzyme activity and inhibition of platelet recruitment. Drosopoulos J H; Broekman M J; Islam N; **Maliszewski C R; Gayle R B 3rd; Marcus A J.** (Department of Medicine, Division of Hematology and Medical Oncology, VA New York Harbor Healthcare System, New York, New York 10010-5050, USA.. jhfliess@mail.med.cornell.edu) . Biochemistry, (2000 Jun 13) 39 (23) 6936-43. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Endothelial cell **CD39**/ecto-ADPase plays a major role in vascular homeostasis. It rapidly metabolizes ADP released from stimulated platelets, thereby preventing further platelet activation and recruitment. We recently developed a recombinant, soluble form of human **CD39**, solCD39, with enzymatic and biological properties identical to **CD39.** To identify amino acids essential for enzymatic/biological activity, we performed site-directed mutagenesis within the four highly conserved apyrase regions of solCD39. Mutation of glutamate 174 to alanine (E174A) and serine 218 to alanine (S218A) resulted in complete and approximately 90% loss of solCD39 enzymatic activity, respectively. Furthermore, compared to wild-type, S57A exhibited a 2-fold increase in ADPase activity without change in ATPase activity, while the tyrosine 127 to alanine (Y127A) mutant lost 50-60% of both ADPase and ATPase activity. The ADPase activity of wild-type solCD39 and each mutant, except for R135A, was greater with calcium as the required divalent cation than with magnesium, but for ATPase activity generally no such preference was observed. Y127A demonstrated the highest calcium/magnesium ADPase activity ratio, 2.8-fold higher than that of wild-type, even though its enzyme activity was greatly reduced. SolCD39 mutants were further characterized by correlating enzymatic with biological activity in an in

vitro platelet aggregation system. Each solCD39 mutant was similar to wild-type in reversing platelet aggregation, except for E174A and S218A. E174A, completely devoid of enzymatic activity, failed to inhibit platelet responsiveness, as anticipated. S218A, with 91% loss of ADPase activity, could still reverse platelet aggregation, albeit much less effectively than wild-type solCD39. Thus, glutamate 174 and serine 218 are essential for both the enzymatic and biological activity of solCD39.

L6 ANSWER 31 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 15

2001:322309 Document No.: PREV200100322309. Identification of functionally important amino acid residues in soluble human **CD39**: An important thrombo-regulator. Drosopoulos, J. H. F. [Reprint author]; Broekman, M. J.; Islam, N.; **Gayle, R. B., III**; **Maliszewski, C. R.**; **Marcus, A. J.**. VA NY Harbor Healthcare System, Weill Medical College of Cornell Univ., New York, NY, USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 813a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Endothelial cell ecto-ADPase/**CD39** plays a major role in maintenance of blood fluidity. It rapidly metabolizes ADP released from activated platelets, thereby preventing further platelet activation and recruitment. We developed a recombinant, soluble form of human **CD39**, solCD39, with enzymatic and biological properties identical to **CD39**. To identify amino acids essential for enzymatic/biological activity, we performed site-directed mutagenesis within the highly conserved apyrase regions (ACR) of solCD39. Mutations E174A and S218A resulted in complete and apprx90% loss of enzymatic activity, respectively. Compared to wild-type, S57A displayed a 2-fold increase in ADPase activity with no change in ATPase activity, whereas Y127A lost apprx55% of both ADPase and ATPase activity. D213A showed the greatest increase in both ADPase and ATPase activity. D54A and D213A had 1.5-fold higher enzyme activity with ATP than with ADP as substrate. Enzymatic activity of solCD39 mutants correlated strongly with their biological activity in an in vitro platelet aggregation system. In citrated plasma, each mutant resembled wild-type in reversing platelet aggregation, with the exception of D54A, E174A, D213A, and S218A. E174A, devoid of enzyme activity, did not inhibit platelet reactivity. S218A, with 91% loss of ADPase activity, could still reverse platelet aggregation, albeit much less effectively than wild-type. Interestingly, D54A and D213A had decreased ability to reverse platelet aggregation, even through their ADPase and ATPase activities were greater than that of wild-type in enzymatic assays. In addition, their ADPase activity in the presence of citrated plasma was also decreased, and this was overcome by addition of excess calcium. The citrate in anticoagulated plasma reduced free calcium to a suboptimal level for full enzymatic activity of D54A and D213A, and decreased their ability to inhibit platelet aggregation as effectively as wild-type solCD39. In heparinized plasma, D54A and D213A completely reversed platelet aggregation and their ADPase activities were similar to that observed in enzyme assays. Kinetic analyses revealed a low binding affinity of D54A and D213A for calcium as well as for ADP and ATP. Decreases in binding were compensated for by increases in rate of catalysis. Thus, aspartates 54 and 213 are involved in calcium binding in the catalytic pocket of solCD39. Glutamate 174 and serine 218 are essential for the enzymatic as well as biological activity of the enzyme. Our study defines amino acid residues required for enzyme catalysis and provides specific information concerning the active site of solCD39 - a potential antithrombotic agent.

L6 ANSWER 32 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:53830 The Genuine Article (R) Number: 367QE. Inhibition of platelet aggregation with **CD39**: An ex vivo dose response study. Buergler

J M (Reprint); Kaluza G L; **Maliszewski C R**; Ali N M. Baylor Coll Med, Houston, TX 77030 USA; Immunex Res & Dev Corp, Seattle, WA 98101 USA. CIRCULATION (31 OCT 2000) Vol. 102, No. 18, Supp. [S], pp. 131-131. MA 630 . ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 33 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:110428 Document No.: PREV200100110428. Inhibition of platelet aggregation with **CD39**: An ex vivo dose response study. Buergler, John M. [Reprint author]; Kaluza, Grzegorz L. [Reprint author]; **Maliszewski, Charles R.**; Ali, Nadir M.. Baylor Coll of Medicine, Houston, TX, USA. Circulation, (October 31, 2000) Vol. 102, No. 18 Supplement, pp. II.131. print.
Meeting Info.: Abstracts from American Heart Association Scientific Sessions 2000. New Orleans, Louisiana, USA. November 12-15, 2000. American Heart Association.
CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

L6 ANSWER 34 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2000:270240 The Genuine Article (R) Number: 290FP. **CD39**/ECTO-adpase blocks and reverses human platelet reactivity: Significance for thrombosis . **Marcus A J (Reprint)**; Drosopoulos J H P; Broekman M J; **Gayle R B**; Islam N; Buergler J; Ali M; **Maliszewski C R.**
Vet Affairs Med Ctr, New York, NY USA; Cornell Univ, Weill Med Coll, New York, NY USA; Baylor Coll Med, Houston, TX 77030 USA; Immunex Corp, Seattle, WA USA. THROMBOSIS AND HAEMOSTASIS (AUG 1999) Supp. [S], pp. 683-684. MA 2161. ISSN: 0340-6245. Publisher: F K SCHATTAUER VERLAG GMBH, P O BOX 10 45 43, LENZHALDE 3, D-70040 STUTTGART, GERMANY. Language: English.

L6 ANSWER 35 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1999:956187 The Genuine Article (R) Number: 250YD. The safety and anti-platelet effects of **CD39** after PTCA in pigs. Buergler J M (Reprint); **Marcus A J**; **Maliszewski C R**; Broekman M J; Schulz D G; Ali N M. Baylor Coll Med, VAMC, Houston, TX 77030 USA; New York Vet Affairs Med Ctr, New York, NY USA; Immunex Res & Dev Corp, Seattle, WA 98101 USA. CIRCULATION (2 NOV 1999) Vol. 100, No. 18, Supp. [S], pp. 472-472. MA 2486. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 36 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1999:956185 The Genuine Article (R) Number: 250YD. **CD39** provides additive inhibition of platelet aggregation over aspirin and abciximab. Buergler J M (Reprint); Kaluza G L; **Maliszewski C R**; Ali N M. Baylor Coll Med, VAMC, Houston, TX 77030 USA; Baylor Coll Med, Methodist Hosp, Houston, TX 77030 USA; Immunex Res & Dev Corp, Seattle, WA 98101 USA . CIRCULATION (2 NOV 1999) Vol. 100, No. 18, Supp. [S], pp. 472-472. MA 2484. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 37 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 16

2000:45209 Document No.: PREV200000045209. Blockade and reversal of human platelet reactivity by **CD39**/ecto-ADPase. Potential for antithrombotic therapeusis. **Marcus, A. J.** [Reprint author]; Broekman, M. J. [Reprint author]; Drosopoulos, J. H. [Reprint author]; **Gayle, R. B., III**; McTaggart, R. A.; Pinsky, D. J.; Islam, N. [Reprint author]; Buergler, J. M.; Ali, M. N.; **Maliszewski, C. R.** . Medicine Hem/Onc, VA NY Harbor Health Care, New York, NY, USA. Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 368a. print.

Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology. New Orleans, Louisiana, USA. December 3-7, 1999. The American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

L6 ANSWER 38 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
1999:955422 The Genuine Article (R) Number: 250YD. Cerebroprotective role of **CD39** (endothelial EctoADPase) in murine stroke. McTaggart R A (Reprint); Broekman J; Peschon J; Stocking K; Choudhri T F; Kim L J; Connolly E S; Drosopoulos J H F; **Maliszewski C R**; **Marcus A J**; Pinsky D J. Columbia Univ, New York, NY USA; Immunex Res & Dev Corp, Seattle, WA 98101 USA; Cornell Univ, New York, NY USA. CIRCULATION (2 NOV 1999) Vol. 100, No. 18, Supp. [S], pp. 328-328. MA 1720. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L6 ANSWER 39 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2000:135870 Document No.: PREV200000135870. **CD39**/ecto-ADPase blocks and reverses human platelet reactivity. Significance for thrombosis. **Marcus, Aaron J.** [Reprint author]; Drosopoulos, Joan H. F.; Broekman, M. Johan; **Gayle, Richard B., III**; Islam, Naziba; Buergler, J.; Ali, M. N.; **Maliszewski, Charles R.**. VA New York Harbor Health Care System, 423 East 23rd Street, New York, NY, 10010, USA. Prostaglandins and Other Lipid Mediators, (Dec., 1999) Vol. 59, No. 1-6, pp. 73. print.
Meeting Info.: 6th International Conference on Eicosanoids and other Bioactive Lipids in Cancer, Inflammation, and Related Diseases. Boston, Massachusetts, USA. September 12-15, 1999.
ISSN: 1098-8823. Language: English.

L6 ANSWER 40 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
1999:769177 The Genuine Article (R) Number: 238PX. **CD39** provides additive inhibition of platelet aggregation over aspirin and abciximab.. Buergler J M (Reprint); **Maliszewski C**; Kaluza G; Kleiman N; Cozart J; Ali M N. Immunex Corp, Seattle, WA USA; Vet Affairs Med Ctr, Houston, TX 77030 USA; Baylor Coll Med, Houston, TX 77030 USA. AMERICAN JOURNAL OF CARDIOLOGY (22 SEP 1999) Vol. 84, No. 6A, Supp. [S], pp. 67P-68P. ISSN: 0002-9149. Publisher: EXCERPTA MEDICA INC, 650 AVENUE OF THE AMERICAS, NEW YORK, NY 10011 USA. Language: English.

L6 ANSWER 41 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2000:37019 Document No.: PREV200000037019. Cerebroprotective role of **CD39** (endothelial ectoADPase) in murine stroke. McTaggart, Ryan A. [Reprint author]; Broekman, M. Johan; Peschon, Jacques; Stocking, Kim; Choudhri, Tanvir F.; Kim, Louis J.; Connolly, E. Sander, Jr.; Drosopoulos, Joan H. F.; **Maliszewski, Charles R.**; **Marcus, Aaron J.**; Pinsky, David J.. Columbia Univ, New York, NY, USA. Circulation, (Nov. 2, 1999) Vol. 100, No. 18 SUPPL., pp. I.328. print.
Meeting Info.: 72nd Scientific Sessions of the American Heart Association. Atlanta, Georgia, USA. November 7-10, 1999.
CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

L6 ANSWER 42 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2000:22278 Document No.: PREV200000022278. The safety and anti-platelet effects of **CD39** after PTCA in pigs. Buergler, John M. [Reprint author]; **Marcus, Aaron J.**; **Maliszewski, Charlie R.**; Broekman, M. Johan; Schulz, Daryl G.; Ali, Nadir M.. Baylor Coll of Medicine, Houston, TX, USA. Circulation, (Nov. 2, 1999) Vol. 100, No. 18 SUPPL., pp. I.472. print.
Meeting Info.: 72nd Scientific Sessions of the American Heart Association.

Atlanta, Georgia, USA. November 7-10, 1999.
CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

L6 ANSWER 43 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2000:22276 Document No.: PREV200000022276. **CD39** provides additive inhibition of platelet aggregation over aspirin and abciximab. Buergler, John M. [Reprint author]; Kaluza, Grzegorz L.; **Maliszewski, Charles R.**; Ali, Nadir M.. Baylor Coll of Medicine, Houston, TX, USA. Circulation, (Nov. 2, 1999) Vol. 100, No. 18 SUPPL., pp. I.472. print. Meeting Info.: 72nd Scientific Sessions of the American Heart Association. Atlanta, Georgia, USA. November 7-10, 1999.
CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

L6 ANSWER 44 OF 54 MEDLINE on STN DUPLICATE 17
1998244993. PubMed ID: 9576748. Inhibition of platelet function by recombinant soluble ecto-ADPase/**CD39**. **Gayle R B 3rd**; **Maliszewski C R**; Gimpel S D; Schoenborn M A; Caspary R G; Richards C; Brasel K; Price V; Drosopoulos J H; Islam N; Alyonycheva T N; Broekman M J; **Marcus A J.** (Immunex Corporation, Seattle, Washington 98101, USA.. gayler@immunex.com) . Journal of clinical investigation, (1998 May 1) 101 (9) 1851-9. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB Excessive platelet accumulation and recruitment, leading to vessel occlusion at sites of vascular injury, present major therapeutic challenges in cardiovascular medicine. Endothelial cell **CD39**, an ecto-enzyme with ADPase and ATPase activities, rapidly metabolizes ATP and ADP released from activated platelets, thereby abolishing recruitment. Therefore, a soluble form of **CD39**, retaining nucleotidase activities, would constitute a novel antithrombotic agent. We designed a recombinant, soluble form of human **CD39**, and isolated it from conditioned media from transiently transfected COS-1 cells and from stably transfected Chinese hamster ovary (CHO) cells. Conditioned medium from CHO cells grown under serum-free conditions was subjected to anti-**CD39** immunoaffinity column chromatography, yielding a single approximately 66-kD protein with ATPase and ADPase activities. Purified soluble **CD39** blocked ADP-induced platelet aggregation in vitro, and inhibited collagen-induced platelet reactivity. Kinetic analyses indicated that, while soluble **CD39** had a Km for ADP of 5.9 microM and for ATP of 2.1 microM, the specificity constant kcat/Km was the same for both substrates. Intravenously administered soluble **CD39** remained active in mice for an extended period of time, with an elimination phase half-life of almost 2 d. The data indicate that soluble **CD39** is a potential therapeutic agent for inhibition of platelet-mediated thrombotic diatheses.

L6 ANSWER 45 OF 54 MEDLINE on STN DUPLICATE 18
1998399871. PubMed ID: 9730622. Gene structure and chromosome location of mouse **Cd39** coding for an ecto-apyrase. Schoenborn M A; Jenkins N A; Copeland N G; Gilbert D J; **Gayle R B 3rd**; **Maliszewski C R.** (Immunex Corporation, Seattle, WA, USA.. schoenborn@immunex.com) . Cytogenetics and cell genetics, (1998) 81 (3-4) 287-9. Journal code: 0367735. ISSN: 0301-0171. Pub. country: Switzerland. Language: English.

L6 ANSWER 46 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 19
1998:384932 Document No.: PREV199800384932. Endothelial cell **CD39** /ecto-ADPASE, a novel platelet inhibitor. **Marcus, A. J.** [Reprint author]; Broekman, M. J.; Drosopoulos, J. H. F.; Islan, N.; Schoenborn, M. A.; Gimpel, S. D.; **Gayle, R. B.**; **Maliszewski, C. R.**. Dep. Veterans Affairs, Cornell Med. Coll., New York, NY, USA. Journal of Investigative Medicine, (March, 1998) Vol. 46, No. 3, pp. 232A. print. Meeting Info.: Annual Meeting of the Association of American Physicians, American Society for Clinical Investigation, American Federation for Medical Research 1998 Biomedicine: Medical Research from Bench to Bedside.

Washington, D.C., USA. May 1-3, 1998. American Federation for Medical Research; American Society for Clinical Investigation; Association of American Physicians.
ISSN: 1081-5589. Language: English.

L6 ANSWER 47 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 20
1999:96018 Document No.: PREV199900096018. Characterization of the structure and function of **CD39. Gayle, R. B., III** [Reprint author]; Gimpel, S. D. [Reprint author]; **Maliszewski, C. R.** [Reprint author]; Schoenborn, M. A. [Reprint author]; Caspary, R. G. [Reprint author]; Dubose, R. F. [Reprint author]; Ketchem, R. R. [Reprint author]; Johnson, R. S. [Reprint author]; Wallace, A. R. [Reprint author]; Drosopoulos, J. H. F. [Reprint author]; Islam, N. [Reprint author]; Broekman, M. J. [Reprint author]; **Marcus, A. J.**. Immunex Corp., Seattle, WA, USA. Blood, (Nov. 15, 1998) Vol. 92, No. 10 SUPPL. 1 PART 1-2, pp. 172A-173A. print.
Meeting Info.: 40th Annual Meeting of the American Society of Hematology. Miami Beach, Florida, USA. December 4-8, 1998. The American Society of Heamatology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

L6 ANSWER 48 OF 54 MEDLINE on STN DUPLICATE 21
97232270. PubMed ID: 9077545. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is **CD39. Marcus A J** ; Broekman M J; Drosopoulos J H; Islam N; Alyonycheva T N; Safier L B; Hajjar K A; Posnett D N; Schoenborn M A; Schooley K A; **Gayle R B** ; **Maliszewski C R.** (Department of Medicine, Veterans Affairs Medical Center, New York 10010-5050, USA.. ajmarcus@mail.med.cornell.edu) . Journal of clinical investigation, (1997 Mar 15) 99 (6) 1351-60.
Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States.
Language: English.
AB We previously demonstrated that when platelets are in motion and in proximity to endothelial cells, they become unresponsive to agonists (Marcus, A.J., L.B. Safier, K.A. Hajjar, H.L. Ullman, N. Islam, M.J. Broekman, and A.M. Eiroa. 1991. J. Clin. Invest. 88:1690-1696). This inhibition is due to an ecto-ADPase on the surface of endothelial cells which metabolizes ADP released from activated platelets, resulting in blockade of the aggregation response. Human umbilical vein endothelial cells (HUVEC) ADPase was biochemically classified as an E-type ATP-diphosphohydrolase. The endothelial ecto-ADPase is herein identified as **CD39**, a molecule originally characterized as a lymphoid surface antigen. All HUVEC ecto-ADPase activity was immunoprecipitated by monoclonal antibodies to **CD39**. Surface localization of HUVEC **CD39** was established by confocal microscopy and flow cytometric analyses. Transfection of COS cells with human **CD39** resulted in both ecto-ADPase activity as well as surface expression of **CD39**. PCR analyses of cDNA obtained from HUVEC mRNA and recombinant human **CD39** revealed products of the same size, and of identical sequence. Northern blot analyses demonstrated that HUVEC express the same sized transcripts for **CD39** as MP-1 cells (from which **CD39** was originally cloned). We established the role of **CD39** as a prime endothelial thromboregulator by demonstrating that **CD39**-transfected COS cells acquired the ability to inhibit ADP-induced aggregation in platelet-rich plasma. The identification of HUVEC ADPase/**CD39** as a constitutively expressed potent inhibitor of platelet reactivity offers new prospects for antithrombotic therapeusis.

L6 ANSWER 49 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
1997:241326 Document No.: PREV199799540529. Antithrombotic activity of human endothelial cell ecto-ADPase/**CD39. Marcus, A.** [Reprint author]; Broekman, M.; Drosopoulos, J.; Islam, N.; Alyonycheva, T.; Hajjar, K.; Posnett, D.; Schoenborn, M.; Schooley, K.; **Gayle, R.**

; **Maliszewski, C.**. Dep. Med., DVA Med. Cent., New York, NY, USA. Journal of Investigative Medicine, (1997) Vol. 45, No. 3, pp. 214A. Meeting Info.: Annual Meeting of the Association of American Physicians, the American Society for Clinical Investigation, and the American Federation for Medical Research: Biomedicine '97 Medical Research from Bench to Bedside. Washington, D.C., USA. April 25-27, 1997. ISSN: 1081-5589. Language: English.

L6 ANSWER 50 OF 54 CAPLUS COPYRIGHT 2005 ACS on STN

1997:626537 Document No. 127:290941 Control of platelet reactivity by an ecto-ADPase on human endothelial cells. **Marcus, A. J.**; Broekman, M. J.; Drosopoulos, J. H. F.; Islam, N.; Alyonycheva, T.; Safier, L. B.; Hajjar, K. A.; Posnett, D. N.; Schoenborn, M. A.; Schooley, K.; **Maliszewski, C. R.** (Div. Hematology/Oncology, Dep. Med., Dep. Veterans Affairs Med. Cent., Division Hematology/Oncology, Dep. Med., Ped., Pathol., Cornell Univ. Med. Coll., New York, NY, USA). Ecto-ATPases: Recent Progress on Structure and Function, [Proceedings of the International Workshop on Ecto-ATPases]. 1st, Mar del Plata, Argentina, Aug. 26-30, 1996, Meeting Date 1996, 167-170. Editor(s): Plesner, Liselotte; Kirley, Terence L.; Knowles, Aileen F. Plenum: New York, N. Y. (English) 1997. CODEN: 65DBAR.

AB A review, with 15 refs., on thromboregulation, biochem. properties of human umbilical vein endothelial cell (HUVEC) ecto-ADPase, and HUVEC ecto-ADPase/**CD39.**

L6 ANSWER 51 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1997:624028 The Genuine Article (R) Number: XE898. Inhibition of platelet reactivity by human endothelial cell ecto-ADPase/**CD39.** **Marcus A J (Reprint)**; Broekman M J; Drosopoulos J H F; Islam N; Alyonycheva T; Safier L B; Hajjar K A; Posnett D N; Schoenborn M A; Schooley K; **Maliszewski C R.** IMMUNEX CORP, SEATTLE, WA; CORNELL UNIV MED COLL, DEPT PEDIAT, NEW YORK, NY; CORNELL UNIV MED COLL, DEPT PATHOL, NEW YORK, NY; CORNELL UNIV MED COLL, DVA MED CTR, DEPT MED, DIV HEMATOL ONCOL, NEW YORK, NY. THROMBOSIS AND HAEMOSTASIS (JUN 1997) Supp. [S], pp. SC15-SC15. ISSN: 0340-6245. Publisher: F K SCHATTAUER VERLAG GMBH , P O BOX 10 45 45, LENZHALDE 3, D-70040 STUTTGART, GERMANY. Language: English.

L6 ANSWER 52 OF 54 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1996:858564 The Genuine Article (R) Number: VT983. **CD39** is the endothelial cell ecto-ADPpase responsible for inhibition of platelet function.. **Marcus A J (Reprint)**; Broekman M J; Drosopoulos J H F; Islam N; Alyonycheva T; Safier L B; Hajjar K A; Posnett D N; Schoenborn M A; Schooley K; **Maliszewski C R.** CORNELL UNIV MED COLL, DEPT VET AFFAIRS MED CTR, DEPT MED, DIV HEMATOL ONCOL, SEATTLE, WA; CORNELL UNIV MED COLL, DEPT PEDIAT, SEATTLE, WA; CORNELL UNIV MED COLL, DEPT PATHOL, SEATTLE, WA; IMMUNEX CORP, SEATTLE, WA. BLOOD (15 NOV 1996) Vol. 88, No. 10, Part 1, Supp. [1], pp. 1850-1850. ISSN: 0006-4971. Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399. Language: English.

L6 ANSWER 53 OF 54 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

1997:54840 Document No.: PREV199799354043. **CD39** is the endothelial cell ecto-ADPase responsible for inhibition of platelet function. **Marcus, A. J.**; Broekman, M. J.; Drosopoulos, J. H. F.; Islam, N.; Alyonycheva, T.; Safier, L. B.; Haijar, K. A; Posnett, D. N.; Schoenborn, M. A.; Schooley, K.; Malizewski, C. R.. Div. Hem./Oncol., Dep. Med., Cornell Univ. Med. Coll., New York, NY, USA. Blood, (1996) Vol. 88, No. 10 SUPPL. 1 PART 1-2, pp. 465A.
Meeting Info.: Thirty-eighth Annual Meeting of the American Society of Hematology. Orlando, Florida, USA. December 6-10, 1996.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

L6 ANSWER 54 OF 54 MEDLINE on STN DUPLICATE 22
95015846. PubMed ID: 7930580. The **CD39** lymphoid cell activation antigen. Molecular cloning and structural characterization. **Maliszewski C R**; Delespesse G J; Schoenborn M A; Armitage R J; Fanslow W C; Nakajima T; Baker E; Sutherland G R; Poindexter K; Birks C; +. (Immunex Research and Development Corporation, Seattle, WA 98101.) Journal of immunology (Baltimore, Md. : 1950), (1994 Oct 15) 153 (8) 3574-83. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB **CD39**, a 70- to 100-kDa molecule expressed primarily on activated lymphoid cells, was previously shown to mediate B cell homotypic adhesion when ligated with a subset of anti-**CD39** mAbs. In the present study, we describe the cloning and molecular characterization of human and murine **CD39**. The nucleotide sequence of human **CD39** includes an open reading frame encoding a putative 510 amino acid protein with six potential N-linked glycosylation sites, 11 Cys residues, and two potential transmembrane regions. Murine **CD39** shares 75% amino acid sequence identity with human **CD39** but fails to cross-react with anti-human **CD39** mAbs. Although there were no significant similarities with other mammalian genes, considerable homology was found between **CD39** and a guanosine diphosphatase from yeast. A series of mouse-human hybrid molecules was constructed to determine the general topology of **CD39** and the location of a biologically functional epitope. These findings and supporting evidence from an anti-**CD39** mAb-selected phage peptide display library indicate a likely model wherein a short intracellular N-terminus is followed by a large extracellular loop containing the epitope recognized by stimulatory anti-**CD39** mAbs, and a short intracellular C terminus. The results demonstrate that **CD39** is a novel cell surface glycoprotein with unusual structural characteristics.

=> s CD39
L8 1355 CD39

=> s l8 and fusion
L9 26 L8 AND FUSION

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10 24 DUP REMOVE L9 (2 DUPLICATES REMOVED)

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L10 ANSWER 1 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
2005:203431 Document No. 142:259992 Gene expression profile in activated CD4-positive T cells useful for the diagnosis and treatment of immune-related diseases. Abbas, Alexander; Clark, Hilary; Ouyang, Wenjun; Williams, Mickey P.; Wood, William I.; Wu, Thomas D. (Genentech, Inc., USA). PCT Int. Appl. WO 2005019258 A2 20050303, 158 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-XA25788 20040810. PRIORITY: US 2003-2003/PV49354W 20030811; WO 2004-US25788 20040810.

AB The present invention relates to composition containing novel proteins and method of using those compns. for the diagnosis and treatment of immune-related diseases. Microarray anal. of human CD4-pos. T-cells activated with an

anti-CD23 and anti-CD28 antibodies together with specific cytokines provides 3232 genes that are differentially expressed in comparison to resting CD4-pos. T-cells. [This abstract record is one of two records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.].

L10 ANSWER 2 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
2005:203433 Document No. 142:259993 Gene expression profile in activated CD4-positive T cells useful for the diagnosis and treatment of immune-related diseases. Abbas, Alexander; Clark, Hilary; Ouyang, Wenjun; Williams, Mickey P.; Wood, William I.; Wu, Thomas D. (Genentech, Inc., USA). PCT Int. Appl. WO 2005016962 A2 20050224, 158 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-XA26249 20040811. PRIORITY: US 2003-2003/PV49354W 20030811; WO 2004-US26249 20040811.
AB The present invention relates to composition containing novel proteins and method of using those compns. for the diagnosis and treatment of immune-related diseases. Microarray anal. of human CD4-pos. T-cells activated with an anti-CD23 and anti-CD28 antibodies together with specific cytokines provides 3232 genes that are differentially expressed in comparison to resting CD4-pos. T-cells. [This abstract record is one of two records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.].

L10 ANSWER 3 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
2004:203617 Document No. 140:247107 Compositions and methods for treating cardiovascular disease. Burton, Paul B.; Deisher, Theresa A. (Immunex Corporation, USA). PCT Int. Appl. WO 2004019866 A2 20040311, 135 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US26354 20030821. PRIORITY: US 2002-2002/PV40641U 20020828; US 2003-2003/PV494457 20030812.
AB The invention pertains to methods of treating cardiovascular disease by modulating inflammatory and immunoregulatory responses associated with such pathol. conditions. Embodiments of the invention provide methods for the treatment of cardiovascular disease in a subject having cardiovascular disease comprising administering an effective amount of one or more IL-17 antagonists, IL-18 antagonists, 4-1BB antagonists, CD30 antagonists, OX40 antagonists and/or **CD39** alone or in any combination.

L10 ANSWER 4 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
2004:759609 Document No. 141:294659 Expression of inflammatory and septic genes to identify antiinflammatory and antiseptic peptides for therapeutic use. Hancock, Robert E. W.; Finlay, B. Brett; Scott, Monisha Gough; Bowdish, Dawn; Rosenberger, Carrie Melissa; Powers, Jon-Paul Steven (Can.). U.S. Pat. Appl. Publ. US 2004180038 A1 20040916, 93 pp., Cont.-in-part of U.S. Pat. Appl. 2004 1,803. (English). CODEN: USXXCO. APPLICATION: US 2003-661471 20030912. PRIORITY: US 2001-2001/PV33663U 20011203; US 2002-2002/308905 20021202.
AB A method of identifying a polynucleotide or pattern of polynucleotides regulated by one or more sepsis or inflammatory inducing agents and

inhibited by a peptide is described. A method of identifying a pattern of polynucleotide expression for inhibition of an inflammatory or septic response. The method includes contacting human epithelial cells with LPS, LTA, CpG DNA and/or intact microbe or microbial components in the presence or absence of a cationic peptide; detecting a pattern of polynucleotide expression for the cells in the presence and absence of the peptide, wherein the pattern in the presence of the peptide represents inhibition of an inflammatory or septic response. Also included are compds. and agents identified by the methods of the invention. In another aspect, the invention provides methods and compds. for enhancing innate immunity in a subject.

L10 ANSWER 5 OF 24 MEDLINE on STN

2003118961. PubMed ID: 12600208. Bacterial expression and characterization of a novel, soluble, calcium-binding, and calcium-activated human nucleotidase. Murphy Deirdre M; Ivanenkov Vasily V; Kirley Terence L. (Department of Pharmacology and Cell Biophysics, College of Medicine, University of Cincinnati, P.O. Box 670575, Cincinnati, Ohio 45267-0575, USA.) Biochemistry, (2003 Mar 4) 42 (8) 2412-21. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB A newly discovered human analogue of a bed bug apyrase, which we named hSCAN-1 for human soluble calcium-activated nucleotidase-1, was expressed in bacteria, refolded from inclusion bodies, purified, and characterized. This apyrase, which is distinct from the eNTPDases exemplified by the endothelial **CD39** (NTPDase1) apyrase, is a 38 kDa monomeric enzyme capable of hydrolyzing a variety of nucleoside di- and triphosphates, but not monophosphates. Preferred substrates include GDP, UDP, and IDP, with a pH optimum for activity between 6 and 7. The specific activity and substrate preference of the bacterially expressed enzyme closely mimic those of the enzyme expressed in mammalian COS cells, as well as the enzyme synthesized in an in vitro bacterial expression system. This suggests that glycosylation and other posttranslational modifications that do not occur in bacteria are not necessary for nucleotidase activity or proper folding of this human apyrase. hSCAN-1 absolutely requires Ca(2+), but not Mg(2+) or other divalent cations analyzed, for enzymatic activity. Surprisingly, the activity does not increase in a quasi-linear fashion at sub-millimolar Ca(2+) concentrations, as would be expected if Ca(2+) were only used as a cosubstrate for the nucleotide substrate, but rather follows a sigmoidal curve. The intrinsic fluorescence and difference absorption studies of hSCAN-1 in the absence of nucleotides revealed Ca(2+)-induced changes in the environment of tryptophan and tyrosine residues with half-saturation at about 90 microM Ca(2+). NaCl increased the half-saturating Ca(2+) concentration needed for both structural changes detected by optical spectroscopy and enzymatic activation of hSCAN-1 detected by nucleotidase assay. These results suggest that Ca(2+) triggers a conformational change in hSCAN-1, converting the enzymatically inactive protein to the active enzyme, in addition to forming the metal-nucleotide substrate complex necessary for nucleotidase activity.

L10 ANSWER 6 OF 24 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:245898 The Genuine Article (R) Number: 652XF. Methylotrophic yeast Pichia pastoris as a host for production of ATP-diphosphohydrolase (apyrase) from potato tubers (Solanum tuberosum). Nourizad N; Ehn M; Gharizadeh B; Hober S; Nyren P (Reprint). Royal Inst Technol, SCFAB, AlbaNova Univ Ctr, Dept Biotechnol, Roslagstullsbacken 21, SE-10691 Stockholm, Sweden (Reprint); Royal Inst Technol, SCFAB, AlbaNova Univ Ctr, Dept Biotechnol, SE-10691 Stockholm, Sweden. PROTEIN EXPRESSION AND PURIFICATION (FEB 2003) Vol. 27, No. 2, pp. 229-237. ISSN: 1046-5928. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB ATP-diphosphohydrolase (apyrase) catalyzes the hydrolysis of phosphoanhydride bonds of nucleoside tri- and di-phosphates in the

presence of divalent cations. This enzyme has broad substrate specificity for nucleotides, which makes it an ideal enzyme for different biotechnical applications, such as DNA sequencing and platelet-aggregation inhibition. The only commercially available apyrase is isolated from potato tubers. To avoid batch-to-batch variations in activity and quality, we decided to produce a recombinant enzyme. The methylotrophic yeast Pichia pastoris was chosen as an eukaryotic expression host. The coding sequence of potato apyrase, without the signal peptide, was cloned into the YpDC541 vector to create a **fusion** with the alpha-mating secretion signal of Saccharomyces cerevisiae. The gene was placed under the control of the methanol-inducible alcohol oxidase promoter. The YpDC541-apyrase construct was integrated into P. pastoris strain SMD1168. Methanol induction resulted in secretion of apyrase to a level of 1 mg/L. The biologically active recombinant apyrase was purified by hydrophobic interaction and ion exchange chromatography. According to SDS-PAGE and Western blot analysis, the purified enzyme showed to be hyperglycosylated. By enzymatic removal of N-glycans, a single band corresponding to a molecular mass of 48 kDa was detected. The recombinant apyrase was found to function well when it was used in combination with the Pyrosequencing technology.

L10 ANSWER 7 OF 24 CAPLUS
2002:676043 Document No. 137:213257 Increased recovery of active proteins using a reduction/oxidation coupling reagent. Sassenfeld, Helmut M.; Remmele, Richard L., Jr.; McCoy, Rebecca E. (Immunex Corporation, USA). PCT Int. Appl. WO 2002068455 A2 20020906, 38 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US5645 20020222. PRIORITY: US 2001-2001/PV271033 20010223.

AB The invention provides methods of increasing yields of desired conformation of proteins. In particular embodiments, the invention includes contacting prepns. of a recombinant protein with a reduction/oxidation coupling reagent for a time sufficient to increase the relative proportion of a desired configurational isomer. A TNF receptor-Fc **fusion** protein fraction with low TNF binding activity was treated with a redox coupling system of reduced glutathione and glutathione to drive the inactive form to the active conformation.

L10 ANSWER 8 OF 24 CAPLUS
2002:555529 Document No. 137:124199 Antibodies to non-functional P2X7 receptor for diagnosis and treatment of cancers and other conditions. Gidley-Baird, Angus; Barden, Julian Alexander (Intreat Pty Ltd., Australia). PCT Int. Appl. WO 2002057306 A1 20020725, 30 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-AU61 20020117. PRIORITY: AU 2001-2579 20010117; AU 2001-5890 20010622; AU 2001-5891 20010622; AU 2001-7430 20010903; AU 2001-7431 20010903.

AB The invention concerns a wide range of diseases and conditions, including cancers. The invention provides a probe for detection of such a disease or condition. The probe is able to distinguish between functional P2X7 receptors and non-functional P2X7 receptors. The probe can do this in various ways, one of which is detecting change in relation to binding of

ATP (ATP) to the receptors. The invention also provides a method for detecting the disease or condition, using the probe. The invention extends to treatment of the disease or condition, using an antibody, or an epitope capable of generating the antibody, which can distinguish between functional and non-functional P2X7 receptors and bind to the non-functional receptors. Methods of treatment, pharmaceutical compns. and use of the probe and antibody are also included.

L10 ANSWER 9 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
2002:11124 Document No. 136:79766 Inhibitors of platelet activation and recruitment. Maliszewski, Charles Richard; Gayle, Richard Brownley; Price, Virginia Lee; Gimpel, Steven Dean (USA). U.S. Pat. Appl. Publ. US 2002002277 A1 20020103, 78 pp., Cont.-in-part of Appl. No. PCT/US99/22955. (English). CODEN: USXXCO. APPLICATION: US 2001-835147 20010413. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813; WO 1999-US22955 19991013.
AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L10 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
2002:688470 Document No. 137:231340 Human **CD39**-like polypeptides and antibodies for treating platelet-related diseases or thrombosis and for screening antithrombotic agent. Ford, John; Mulero, Julio J.; Yeung, George (Hyseq, Inc., USA). U.S. US 6447771 B1 20020910, 65 pp., Cont.-in-part of Appl. No. PCT/US99/16180. (English). CODEN: USXXAM. APPLICATION: US 1999-370265 19990809. PRIORITY: US 1999-273447 19990319; US 1999-350836 19990709; WO 1999-US16180 19990716.
AB The invention provides polynucleotides isolated from cDNA libraries of human fetal liver-spleen and macrophage as well as polypeptides encoded by these polynucleotides and mutants or variants thereof. The polypeptides correspond to a human **CD39**-like protein. Other aspects of the invention include vectors containing polynucleotides of the invention and related host cells as well a processes for producing **CD39**-like polypeptides, and antibodies specific for such polypeptides. The **CD39**-like proteins, polynucleotides, and antibodies are useful for reducing ADP:ATP ratio; for treating platelet-related diseases, e.g. myocardial infarction and ischemia, thrombosis, platelet aggregation, inflammation and cerebral ischemia; and for identifying therapeutic agents.

L10 ANSWER 11 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
2002:6330 Document No. 136:84678 Methods and materials relating to **CD39**-like polypeptides. Ford, John; Mulero, Julio J.; Yeung, George (Hyseq, Inc., USA). U.S. US 6335013 B1 20020101, 98 pp., Cont.-in-part of U. S. Ser. No 583,231. (English). CODEN: USXXAM. APPLICATION: US 2000-608285 20000630. PRIORITY: US 1999-273447 19990319; US 1999-350836 19990709; WO 1999-US16180 19990716; US 1999-370265 19990809; US 2000-2000/481238 20000111; US 2000-2000/557800 20000425; US 2000-2000/583231 20000526.
AB The invention provides novel polynucleotides isolated from cDNA libraries of human fetal liver-spleen and macrophage as well as polypeptides encoded by these polynucleotides and mutants or variants thereof. The polypeptides correspond to a novel human **CD39**-like protein. Other aspects of the invention include vectors containing polynucleotides of the invention and related host cells as well a processes for producing novel **CD39**-like polypeptides, and antibodies specific for such polypeptides.

L10 ANSWER 12 OF 24 MEDLINE on STN
2002424751. PubMed ID: 12181428. A **fusion** protein of the human P2Y(1) receptor and NTPDase1 exhibits functional activities of the native receptor and ectoenzyme and reduced signaling responses to endogenously released nucleotides. Alvarado-Castillo Claudia; Lozano-Zarain Patricia; Mateo Jesus; Harden T Kendall; Boyer Jose L. (Department of Pharmacology,

University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599, USA.. tkh@med.unc.edu) . Molecular pharmacology, (2002 Sep) 62 (3) 521-8. Journal code: 0035623. ISSN: 0026-895X. Pub. country: United States. Language: English.

AB To begin to address the functional interactions between constitutively released nucleotides, ectonucleotidase activity, and P2Y receptor-promoted signaling responses, we engineered the human P2Y(1) receptor in a **fusion** protein with a member of the ectonucleoside triphosphate diphosphohydrolase family, NTPDase1. Membranes prepared from Chinese hamster ovary (CHO)-K1 cells stably expressing either wild-type NTPDase1 or the P2Y(1) receptor-NTPDase1 **fusion** protein exhibited nucleotide-hydrolytic activities that were over 300-fold greater than activity measured in membranes from empty vector-transfected cells. The molecular ratio for nucleoside triphosphate versus diphosphate hydrolysis was approximately 1:0.4 for both the wild-type NTPDase1 and P2Y(1)-NTPDase1 **fusion** protein. Stable expression of the P2Y(1)-NTPDase1 **fusion** protein conferred an ADP and 2MeSADP-promoted Ca(2+) response to CHO-K1 cells. Moreover, the maximal capacity of the nonhydrolyzable agonist ADPbetaS to stimulate inositol phosphate accumulation was similar, and the EC(50) of ADPbetaS was lower in the **fusion** protein than the wild-type receptor. In contrast, the substantial nucleotide-hydrolyzing activity of the **fusion** protein resulted in a greater than 50-fold shift to the right of the concentration-effect curve of ADP for activation of phospholipase C compared with the wild-type receptor. Heterologous expression of the P2Y(1) and other P2Y receptors results in marked increases in basal inositol phosphate levels. Given the high nucleotidase activity and apparently normal receptor signaling activity of the P2Y(1) receptor-NTPDase1 **fusion** protein, we quantitated basal inositol phosphate accumulation in cells stably expressing either the wild-type P2Y(1) receptor or the **fusion** protein. Although marked elevation of inositol phosphate levels occurred with wild-type P2Y(1) receptor expression, levels in cells expressing the **fusion** protein were not different from those in wild-type CHO-K1 cells.

L10 ANSWER 13 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

2001:283744 Document No. 134:309689 DNA vaccines encoding antigen linked to a domain that binds CD40. Ledbetter, Jeffrey A.; Hayden-Ledbetter, Martha S. (USA). PCT Int. Appl. WO 2001026608 A2 20010419, 55 pp. DESIGNATED STATES: W: AU, CA, CN, JP, MX, NZ, SE, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US28414 20001013. PRIORITY: US 1999-PV159690 19991014.

AB Vaccines that target one or more antigens to a cell surface receptor improve the antigen-specific humoral and cellular immune response. Antigen(s) linked to a domain that binds to a cell surface receptor are internalized, carrying antigen(s) into an intracellular compartment where the antigen(s) are digested into peptides and loaded onto MHC mols. T cells specific for the peptide antigens are activated, leading to an enhanced immune response. The vaccine may comprise antigen(s) linked to a domain that binds at least one receptor or a DNA plasmid encoding antigen(s) linked to a domain that binds at least one receptor. A preferred embodiment of the invention targets HIV-1 env antigen to the CD40 receptor, resulting in delivery of antigen to CD40 pos. cells, and selective activation of the CD40 receptor on cells presenting HIV-1 env antigens to T cells.

L10 ANSWER 14 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

2001:152816 Document No. 134:203413 Veggie-CHO cells genetically engineered for IGF-1 signaling pathway genes for improved cell culture. Morris, Arvia E.; Reddy, Pranhitha (Immunex Corporation, USA). PCT Int. Appl. WO 2001014529 A1 20010301, 49 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP,

KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US23483 20000825. PRIORITY: US 1999-PV150645 19990825; US 1999-PV168948 19991203; US 1999-PV171949 19991223.

AB The invention provides improved methods of recombinant protein production in cell culture based on the discovery that Veggie-CHO cells, which have been adapted over many generations to growth in serum-free and protein-free medium, have alterations in the intracellular IGF-1 (insulin-like growth factor 1) receptor signaling cascade. Advantageous phenotypes of the Veggie-CHO cells can be duplicated in a more controlled, consistent and reliable manner by genetically engineering individual components of the IGF-1 signaling pathways. Preferred IGS-1 signaling pathway genes are a protein kinase B (PKB) gene, a MEK1 or MEK2 gene, a glut5 or glut1 gene, and ERK1 genes, a JNK gene, a 14-3-3 protein gene, a PDK gene, an IRS gene and a phosphatidylinositol 3-kinase gene. Specifically, the invention relates to the modulation of the IGF-1 signaling pathway in cells so as to improve production characteristics.

L10 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

2001:136929 Document No. 134:173037 Method using **CD39**/ecto-ADPase for treating thrombotic and ischemic disorders. Pinsky, David J. (The Trustees of Columbia University in the City of New York, USA). PCT Int. Appl. WO 2001011949 A1 20010222, 119 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US22060 20000811. PRIORITY: US 1999-374586 19990813.

AB The present invention provides a method of treating or preventing thrombotic or ischemic disorders in a subject which comprises administering an **CD39**/ecto-ADPase to the subject, wherein the **CD39**/ecto-ADPase inhibits ADP-mediated platelet aggregation by increasing ADP catabolism, and a method for determining whether a compound inhibits platelet aggregation by increasing ADP catabolism so as to treat or prevent thrombotic or ischemic disorders in a subject, comprising: (a) inducing thrombotic or ischemic disorders in an animal, which animal is an animal model for thrombotic or ischemic disorders; (b) measuring the stroke outcome in said animal; (c) measuring platelet deposition and/or fibrin deposition in ischemic tissue; and (d) comparing the stroke outcome in step (b) and the platelet deposition and/or fibrin deposition with that of the animal model in the absence of the compound so as to identify a compound capable of treating or preventing thrombotic or ischemic disorders in a subject. Also disclosed are human **CD39**/ecto-ADPase sequences.

L10 ANSWER 16 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

2000:368704 Document No. 133:14300 In situ method of analyzing cells by staining with multiple stains and using a spectral data collection device. Garini, Yuval; Mcnamara, George; Soenksen, Dirk G.; Cabib, Dario; Buckwald, Robert A. (Applied Spectral Imaging Ltd., Israel). PCT Int. Appl. WO 2000031534 A1 20000602, 129 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO

1999-US27000 19991116. PRIORITY: US 1998-196690 19981120.

AB A method of in situ anal. of a biol. sample comprises the steps of (a) staining the biol. sample with N stains of which a first stain is selected from the group consisting of a first immunohistochem. stain, a first histol. stain and a first DNA ploidy stain, and a second stain is selected from the group consisting of a second immunohistochem. stain, a second histol. stain and a second DNA ploidy stain, with provisions that N is an integer greater than three and further that (i) if the first stain is the first immunohistochem. stain then the second stain is either the second histol. stain or the second DNA ploidy stain; (ii) if the first stain is the first histol. stain then the second stain is either the second immunohistochem. stain or the second DNA ploidy stain; whereas (iii) if the first stain is the first DNA ploidy stain then the second stain is either the second immunohistochem. stain or the second histol. stain; and (b) using a spectral data collection device for collecting spectral data from the biol. sample, the spectral data collection device and the N stains are selected so that a spectral component associated with each of the N stains is collectible. Figure (1) shows a block diagram illustrating the main components of an imaging spectrometer. Breast cancer tissue samples were stained with two histol. stains (hematoxylin and eosin), and four immunohistochem. stains (DAB, AEC, Fast Red, and BCIP/NBT) and measured using the Spectracube system.

L10 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

2000:277996 Document No. 132:303497 **CD39** polypeptides as inhibitors of platelet activation and recruitment. Maliszewski, Charles R.; Gayle, Richard B., III; Price, Virginia L.; Gimpel, Steven D. (Immunex Corp., USA). PCT Int. Appl. WO 2000023459 A1 20000427, 122 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US22955 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L10 ANSWER 18 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

2000:277866 Document No. 132:303495 Methods of inhibiting platelet activation and recruitment. Maliszewski, Charles R.; Gayle, Richard B., III; Marcus, Aaron J. (Immunex Corp., USA; Cornell Research Foundation, Inc.). PCT Int. Appl. WO 2000023094 A2 20000427, 118 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23641 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L10 ANSWER 19 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

2000:68476 Document No. 132:135506 Identification of novel homologs of **CD39** antigens of human and cDNAs encoding them. Ford, John; Mulero, Julio (Hyseq, Inc., USA). PCT Int. Appl. WO 2000004041 A2

20000127, 125 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US16180 19990716. PRIORITY: US 1998-118205 19980716; US 1998-122449 19980724; US 1999-244444 19990204; US 1999-273447 19990319; US 1999-350836 19990709.

AB CDNAs for homologs of the human **CD39** antigen are cloned from cDNA libraries of human fetal liver-spleen and macrophage and the gene products are characterized. The proteins may be of use in the treatment of clotting disorders including thrombosis. Preliminary clones were obtained from a human fetal liver spleen cDNA library by determination of a sequence signature sequence followed by sequencing of those clones with **CD39**-like signatures. The clone obtained encoded a **CD39** -like protein and hybridized to an mRNA from macrophages, but not from any other tissue tested. Unlike **CD39**, this protein was soluble and secreted from cells and was shown to be an apyrase. It also shared conserved sequences with other apyrases and mutation of the conserved regions affected the apyrase activity.

L10 ANSWER 20 OF 24 MEDLINE on STN

2001069394. PubMed ID: 10954728. Regulation of yeast ectoapyrase ynd1p activity by activator subunit Vma13p of vacuolar H+-ATPase. Zhong X; Malhotra R; Guidotti G. (Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138, USA.) Journal of biological chemistry, (2000 Nov 10) 275 (45) 35592-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **CD39**-like ectoapyrases are involved in protein and lipid glycosylation in the Golgi lumen of Saccharomyces cerevisiae. By using a two-hybrid screen, we found that an activator subunit (Vma13p) of yeast vacuolar H(+)-ATPase (V-ATPase) binds to the cytoplasmic domain of Ynd1p, a yeast ectoapyrase. Interaction of Ynd1p with Vma13p was demonstrated by direct binding and co-immunoprecipitation. Surprisingly, the membrane-bound ADPase activity of Ynd1p in a vma13Delta mutant was drastically increased compared with that of Ynd1p in VMA13 cells. A similar increase in the apyrase activity of Ynd1p was found in a vma1Delta mutant, in which the catalytic subunit A of V-ATPase is missing, and the membrane peripheral subunits including Vma13p are dissociated from the membranes. However, the E286Q mutant of VMA1, which assembles inactive V-ATPase complex including Vma13p in the membrane, retained wild type levels of Ynd1p activity, demonstrating that the presence of Vma13p rather than the function of V-ATPase in the membrane represses Ynd1p activity. These results suggest that association of Vma13p with the cytoplasmic domain of Ynd1p regulates its apyrase activity in the Golgi lumen.

L10 ANSWER 21 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

1999:795994 Document No. 132:31744 Gene probes used for genetic profiling in healthcare screening and planning. Roberts, Gareth Wyn (Genostic Pharma Ltd., UK). PCT Int. Appl. WO 9964627 A2 19991216, 745 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1780 19990604. PRIORITY: GB 1998-12099 19980606; GB 1998-13291 19980620; GB 1998-13611 19980624; GB 1998-13835 19980627; GB 1998-14110 19980701; GB 1998-14580 19980707; GB 1998-15438 19980716; GB 1998-15576 19980718; GB 1998-15574 19980718; GB 1998-16085 19980724; GB 1998-16086 19980724; GB 1998-16921 19980805; GB 1998-17097 19980807; GB 1998-17200 19980808; GB 1998-17632 19980814; GB 1998-17943 19980819.

AB There is considerable evidence that significant factor underlying the individual variability in response to disease, therapy and prognosis lies in a person's genetic make-up. There have been numerous examples relating that polymorphisms within a given gene can alter the functionality of the protein encoded by that gene thus leading to a variable physiol. response. In order to bring about the integration of genomics into medical practice and enable design and building of a technol. platform which will enable the everyday practice of mol. medicine a way must be invented for the DNA sequence data to be aligned with the identification of genes central to the induction, development, progression and outcome of disease or physiol. states of interest. According to the invention, the number of genes and their configurations (mutations and polymorphisms) needed to be identified in order to provide critical clin. information concerning individual prognosis is considerably less than the 100,000 thought to comprise the human genome. The identification of the identity of the core group of genes enables the invention of a design for genetic profiling technologies which comprises of the identification of the core group of genes and their sequence variants required to provide a broad base of clin. prognostic information - "genostics". The "Genostic" profiling of patients and persons will radically enhance the ability of clinicians, healthcare professionals and other parties to plan and manage healthcare provision and the targeting of appropriate healthcare resources to those deemed most in need. The use of this invention could also lead to a host of new applications for such profiling technologies, such as identification of persons with particular work or environment related risk, selection of applicants for employment, training or specific opportunities or for the enhancing of the planning and organization of health services, education services and social services.

L10 ANSWER 22 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

1999:795993 Document No. 132:31743 Gene probes used for genetic profiling in healthcare screening and planning. Roberts, Gareth Wyn (Genostic Pharma Limited, UK). PCT Int. Appl. WO 9964626 A2 19991216, 149 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1779 19990604. PRIORITY: GB 1998-12098 19980606; GB 1998-28289 19981223.

AB There is considerable evidence that significant factor underlying the individual variability in response to disease, therapy and prognosis lies in a person's genetic make-up. There have been numerous examples relating that polymorphisms within a given gene can alter the functionality of the protein encoded by that gene thus leading to a variable physiol. response. In order to bring about the integration of genomics into medical practice and enable design and building of a technol. platform which will enable the everyday practice of mol. medicine a way must be invented for the DNA sequence data to be aligned with the identification of genes central to the induction, development, progression and outcome of disease or physiol. states of interest. According to the invention, the number of genes and their configurations (mutations and polymorphisms) needed to be identified in order to provide critical clin. information concerning individual prognosis is considerably less than the 100,000 thought to comprise the human genome. The identification of the identity of the core group of genes enables the invention of a design for genetic profiling technologies.

L10 ANSWER 23 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

1998:471045 Document No. 129:229466 Effects of explosive brain death on cytokine activation of peripheral organs in the rat. Takada, Moriatsu; Nadeau, Kari C.; Hancock, Wayne W.; Mackenzie, Harald S.; Shaw, Gray D.; Waaga, Ana Marie; Chandraker, Anil; Sayegh, Mohamed H.; Tilney, Nicholas

L. (Surgical Research Laboratory, Harvard Medical School, Boston, MA, USA). Transplantation, 65(12), 1533-1542 (English) 1998. CODEN: TRPLAU. ISSN: 0041-1337. Publisher: Williams & Wilkins.

AB The success rate of transplanted organs from brain-dead cadaver donors is consistently inferior to that of living sources. As cadaver and living unrelated donors are equally genetically disparate with a given recipient, the difference must lie within the donor himself and/or the effects of organ preservation and storage. The authors have hypothesized that irreversible central nervous system injury may up-regulate proinflammatory mediators and cell surface mols. in peripheral organs to be engrafted, making them more prone to host inflammatory and immunol. responses. Rats undergoing surgically induced acutely increased intracranial pressure (explosive brain death) were followed for 6 h. Their peripheral tissues were examined by reverse transcriptase polymerase chain reaction and immunohistol., serum factors were assessed by ELISA, and the influence of inflammatory mols. in the blood stream was determined by cross-circulation expts. with normal animals. MRNA expression of both lymphocyte- and macrophage-associated products increased dramatically in all tissues. Similar factors in serum were co-incidentally increased; these were shown to be active in vivo by cross-circulation with normal animals. The organs of all control groups, including animals with important ischemic injury and with hemorrhagic shock, were neg. Up-regulation of MHC class I and II antigens and the co-stimulatory mol. B7 suggests increased immunogenicity of the peripheral organs. These changes could be inhibited by: (i) administration of a recombinant soluble P-selectin glycoprotein ligand-Ig, a P- and E-selectin antagonist; and (ii) a **fusion** protein, cytotoxic T lymphocyte antigen 4-Ig, which blocks B7-mediated T-cell co-stimulation. Activation of peripheral organs following explosive brain death may be caused by various interrelated events, including the effects of massive acute central injury, hypotension, and circulating factors. Almost complete suppression of these changes could be produced by biol. agents. Such interventions, if reproducible in humans, could improve the quality of organs from "marginal" donors, broadening the criteria for donor acceptance.

L10 ANSWER 24 OF 24 MEDLINE on STN DUPLICATE 1

89195744. PubMed ID: 2539279. Changes in the phenotype and immunoglobulin secretion of human B cells following co-culture with cells of an EBV+ lymphoblastoid line or **fusion** with mouse plasmacytoma cells. Studies in short-term and long-term culture. Ling N R; Lowe J A. (Department of Immunology, University of Birmingham, UK.) Clinical and experimental immunology, (1989 Feb) 75 (2) 311-6. Journal code: 0057202. ISSN: 0009-9104. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The mechanisms controlling immunoglobulin production have been studied in two types of immunoglobulin-secreting cell generated from human B cells. The first type (I) was produced by activation and transformation of B cells by co-culture with cells of an Epstein-Barr-virus-positive (EBV+) lymphoblastoid cell line (EBV-B-LCL). The second type (II) consisted of human/mouse hybrid cells produced by fusing human tonsil B cells with cells of a mouse plasmacytoma line. Both these methods, singly and in combination, have been widely used for initiation of cell lines secreting human monoclonal antibodies (MoAbs). The two cell types were of quite different phenotype with respect to human B cell antigens. In type I cells MHC Class II and the pan B antigens CD19 and CD37 were expressed at levels typical of cells at the B cell stage. The antigens CD23 and **CD39** were expressed at the high levels characteristic of EBV-transformed B cells. Type II cells expressed few B cell antigens. MHC Class II, pan B and the CD23 and **CD39** antigens were very weakly expressed and by 119 days post-**fusion** only CD38 was detectable on cells of the three lines studied; CD9 was on two and CD19 on only one of the three lines. Thus the phenotype of type I cells was influenced by EBV transformation but was otherwise typical of activated B cells. Whereas the human B antigen expression of the hybrid (type II) cells was at the low level encountered on human plasma cells. It is suggested that **fusion** of a human B cell to a mouse cell which is

at the plasmacytoid stage of differentiation results in a switching off of the expression of human peripheral B antigens by a differentiation-linked mechanism. These results are considered in relation to the practical aspects of the production of human MoAbs and the theoretical aspects of control of the passage of B cells to a secretory stage of differentiation.

=> s CD39 fusion
L11 0 CD39 FUSION

=> s CD39 chimera
L12 0 CD39 CHIMERA

=> s soluble CD39
L13 51 SOLUBLE CD39

=> s l13 and platelet activation
L14 14 L13 AND PLATELET ACTIVATION

=> dup remove l14
PROCESSING COMPLETED FOR L14
L15 9 DUP REMOVE L14 (5 DUPLICATES REMOVED)

=> d l15 1-9 cbib abs

L15 ANSWER 1 OF 9 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2005203205 EMBASE Role of CD39 (NTPDase-1) in thromboregulation, cerebroprotection, and cardioprotection. Marcus A.J.; Broekman M.J.; Drosopoulos J.H.F.; Olson K.E.; Islam N.; Pinsky D.J.; Levi R.. Dr. A.J. Marcus, Hematology/Oncology, VA New York Harbor Healthcare System, Weill Med. Coll. of Cornell Univ., 423 East 23rd Street, New York, NY 10010, United States. ajmarcus@med.cornell.edu. Seminars in Thrombosis and Hemostasis Vol. 31, No. 2, pp. 234-246 2005.
Refs: 70.
ISSN: 0094-6176. CODEN: STHMBV
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20050602
AB Blood platelets maintain vascular integrity and promote primary and secondary hemostasis following interruption of vessel continuity. Biochemical or physical damage to coronary, carotid, or peripheral arteries promotes excessive **platelet activation** and recruitment culminating in vascular occlusion and tissue ischemia. Currently, inadequate therapeutic approaches to stroke and coronary artery disease (CAD) are a public health issue. Following our demonstration of neutrophil leukotriene production from arachidonate released from activated aspirin-treated platelets, we studied interactions among platelets and other blood cells. This led to concepts of transcellular metabolism and thromboregulation. Thrombosis has a proinflammatory component whereby biologically active substances are synthesized by different cell types that could not individually synthesize the metabolite(s). Endothelium controls platelet reactivity via at least three biochemical systems: autacoids leading to production of prostacyclin and nitric oxide (NO) and endothelial ecto-adenosine phosphatase (ADPase)/CD39/nucleoside triphosphate diphosphohydrolase (NTPDase-1). The autacoids are fluid phase reactants, not produced by tissues in the basal state, but are only synthesized intracellularly and released upon interactions of cells with an agonist. When released, they exert fleeting actions in the immediate milieu and are rapidly inactivated. CD39 is an integral component of the endothelial cell (EC) surface and is substrate activated. It maintains vascular fluidity in the complete absence of prostacyclin and NO, indicating that the latter are ancillary components of hemostasis. Therapeutic implications for the autacoids have not been compelling because of their transient and local action and limited potency. Conversely, CD39, acting solely on the platelet releasate, is

efficacious in animal models. It metabolically neutralizes a prothrombotic releasate via deletion of ADP-the major recruiting agent responsible for formation of an occlusive thrombus. In addition, solCD39 reduced adenosine triphosphate (ATP)- and ischemia-induced norepinephrine release in the heart. This action can prevent fatal arrhythmia. Moreover, solCD39 ameliorated the sequelae of stroke in cd39-null mice. Thus, CD39 represents the next generation of cardioprotective and cerebroprotective molecules. This article focuses on our interpretations of recent data and their implications for therapeutics. Copyright .COPYRGT. 2005 by Thieme Medical Publishers, Inc.

L15 ANSWER 2 OF 9 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 1

2003111233 EMBASE Neuroprotective effect of SolCD39, a novel platelet aggregation inhibitor, on transient middle cerebral artery occlusion in rats. Belayev L.; Khoutorova L.; Deisher T.A.; Belayev A.; Busto R.; Zhang Y.; Zhao W.; Ginsberg M.D.. Dr. L. Belayev, Department of Neurology (D4-5), Univ. of Miami School of Medicine, PO Box 016960, Miami, FL 33101, United States. lbelayev@stroke.med.miami.edu. Stroke Vol. 34, No. 3, pp. 758-763 1 Mar 2003.
Refs: 29.
ISSN: 0039-2499. CODEN: SJCCA7
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20030327

AB Background and Purpose - SolCD39 is a soluble form of recombinant human ecto-ATP/ADPase (NTPDasel) and represents a new class of antithrombotic agents. SolCD39 blocks and reverses **platelet activation**, preventing recruitment of additional platelets into a growing thrombus. The purpose of this study was to examine the effect of solCD39 on neurological deficit, infarct size, and extent of edema after transient middle cerebral artery occlusion (MCAO) in rats. Methods - Physiologically controlled Sprague-Dawley rats underwent 2-hour MCAO by retrograde insertion of an intraluminal suture coated with poly-L-lysine. The agent (solCD39) was administered intravenously before MCAO or at 1-hour or 3-hour recirculation. Other groups received vehicle (Tris-buffered saline) or human albumin (as a "positive" neuroprotective control; 25%, 0.5% of body weight) at 1-hour recirculation. Neurological status was evaluated during occlusion (at 60 minutes) and daily for 3 days after MCAO. Brains were perfusion-fixed at 72 hours, and infarct volumes and brain swelling were determined. Results - Pretreatment with solCD39 significantly improved the neurological score at 72 hours compared with the vehicle group (4.4±0.6 versus 7.6±0.6, respectively; P=0.008). Cortical infarct areas were significantly reduced at multiple levels by pretreatment with solCD39. Total striatal infarct area was also significantly reduced compared with vehicle by both solCD39 pretreatment (48% mean reduction) and solCD39 treatment at 3-hour recirculation (51.% mean reduction). Treatment with SolCD39 significantly reduced total infarct volume (corrected for brain swelling) by an average of 71% to 72% when administered either before ischemia or at 3 hours of recirculation compared with vehicle. Treatment with albumin significantly reduced neurological score and total, cortical, and subcortical infarction at multiple levels, as expected. Conclusions - Treatment with SolCD39, administered either before or at 3 hours after MCAO, improves neurological score and reduces infarct size compared with vehicle. A pharmacological agent of this type appears to have potential for the treatment of focal ischemic stroke.

L15 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN

2003:262463 Document No. 139:206845 Metabolic control of excessive extracellular nucleotide accumulation by CD39/ecto-nucleotidase-1: Implications for ischemic vascular diseases. Marcus, Aaron J.; Broekman, M. Johan; Drosopoulos, Joan H. F.; Islam, Naziba; Pinsky, David J.; Sesti, Casilde; Levi, Roberto (Department of Medicine, Weill Medical College of Cornell University, New York, NY, USA). Journal of Pharmacology and Experimental Therapeutics, 305(1), 9-16 (English) 2003. CODEN: JPETAB.

ISSN: 0022-3565. Publisher: American Society for Pharmacology and Experimental Therapeutics.

AB A review. Platelets are responsible for maintaining vascular integrity. In thrombocytopenic states, vascular permeability and fragility increase, presumably due to the absence of this platelet function. Chemical or phys. injury to a blood vessel induces **platelet activation** and platelet recruitment. This is beneficial for the arrest of bleeding (hemostasis), but when an atherosclerotic plaque is ulcerated or fissured, it becomes an agonist for vascular occlusion (thrombosis). Expts. in the late 1980s cumulatively indicated that endothelial cell CD39-an ecto-ADPase-reduced platelet reactivity to most agonists, even in the absence of prostacyclin or nitric oxide. As discussed herein, CD39 rapidly and preferentially metabolizes ATP and ADP released from activated platelets to AMP, thereby drastically reducing or even abolishing platelet aggregation and recruitment. Since ADP is the final common agonist for platelet recruitment and thrombus formation, this finding highlights the significance of CD39. A recombinant, soluble form of human CD39, solCD39, has enzymic and biol. properties identical to the full-length form of the mol. and strongly inhibits human platelet aggregation induced by ADP, collagen, arachidonate, or TRAP (thrombin receptor agonist peptide). In sympathetic nerve endings isolated from guinea pig hearts, where neuronal ATP enhances norepinephrine exocytosis, solCD39 markedly attenuated norepinephrine release. This suggests that NTPDase (nucleoside triphosphate diphosphohydrolase) could exert a cardioprotective action by reducing ATP-mediated norepinephrine release, thereby offering a novel therapeutic approach to myocardial ischemia and its consequences. In a murine model of stroke, driven by excessive platelet recruitment, solCD39 reduced the sequelae of stroke, without an increase in intracerebral hemorrhage. CD39 null mice, generated by deletion of apyrase-conserved regions 2 to 4, exhibited a decrease in postischemic perfusion and an increase in cerebral infarct volume when compared with controls. "Reconstitution" of CD39 null mice with solCD39 reversed these changes. We hypothesize that solCD39 has potential as a novel therapeutic agent for thrombotic diatheses.

L15 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN

2002:11124 Document No. 136:79766 Inhibitors of **platelet activation** and recruitment. Maliszewski, Charles Richard; Gayle, Richard Brownley; Price, Virginia Lee; Gimpel, Steven Dean (USA). U.S. Pat. Appl. Publ. US 2002002277 A1 20020103, 78 pp., Cont.-in-part of Appl. No. PCT/US99/22955. (English). CODEN: USXXCO. APPLICATION: US 2001-835147 20010413. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813; WO 1999-US22955 19991013.

AB The present invention provides **soluble CD39** polypeptides and compns., and methods for inhibiting **platelet activation** and recruitment in a mammal comprising administering a **soluble CD39** polypeptide.

L15 ANSWER 5 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:807370 The Genuine Article (R) Number: 600GK. Roles of Asp54 and Asp213 in Ca2+ utilization by soluble human CD39/ecto-nucleotidase. Drosopoulos J H F (Reprint). VA New York Harbor Healthcare Syst, Thrombosis Res Lab, Res Serv, Room 13026W, 423 E 23rd St, New York, NY 10010 USA (Reprint); VA New York Harbor Healthcare Syst, Thrombosis Res Lab, Res Serv, New York, NY 10010 USA; Cornell Univ, Weill Med Coll, Dept Med, Div Hematol & Med Oncol, New York, NY 10010 USA. ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS (1 OCT 2002) Vol. 406, No. 1, pp. 85-95. ISSN: 0003-9861. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Soluble human CD39 (solCD39) rapidly metabolizes nucleotides, especially ADP released from activated platelets, thereby inhibiting further **platelet activation** and recruitment. Using

alanine substitution mutagenesis, we established a functional role for aspartates D54 and D213 in solCD39. Kinetic analyses of D54A and D213A indicated decreased K(m)s of the mutants, compared to wild type, for the cofactor calcium and for the substrates ADP and ATP. These decreases in calcium and nucleotide affinity of the mutants were accompanied by increases in their rate of catalysis. The decreased affinity of the mutants for calcium was responsible for their diminished ability to reverse platelet aggregation in plasma anticoagulated with citrate, a known calcium chelator. Their ADPase activity in the presence of citrated plasma was also decreased, although this could be overcome with excess calcium. Thus, aspartates 54 and 213 are involved in calcium utilization and potentially involved in cation coordination with substrate in the catalytic pocket of solCD39.

L15 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
2000:277996 Document No. 132:303497 CD39 polypeptides as inhibitors of **platelet activation** and recruitment. Maliszewski, Charles R.; Gayle, Richard B., III; Price, Virginia L.; Gimpel, Steven D. (Immunex Corp., USA). PCT Int. Appl. WO 2000023459 A1 20000427, 122 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US22955 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

AB The present invention provides **soluble CD39** polypeptides and compns., and methods for inhibiting **platelet activation** and recruitment in a mammal comprising administering a **soluble CD39** polypeptide.

L15 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
2000:277866 Document No. 132:303495 Methods of inhibiting **platelet activation** and recruitment. Maliszewski, Charles R.; Gayle, Richard B., III; Marcus, Aaron J. (Immunex Corp., USA; Cornell Research Foundation, Inc.). PCT Int. Appl. WO 2000023094 A2 20000427, 118 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23641 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

AB The present invention provides **soluble CD39** polypeptides and compns., and methods for inhibiting **platelet activation** and recruitment in a mammal comprising administering a **soluble CD39** polypeptide.

L15 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 2
2000302517. PubMed ID: 10841775. Site-directed mutagenesis of human endothelial cell ecto-ADPase/**soluble CD39**: requirement of glutamate 174 and serine 218 for enzyme activity and inhibition of platelet recruitment. Drosopoulos J H; Broekman M J; Islam N; Maliszewski C R; Gayle R B 3rd; Marcus A J. (Department of Medicine, Division of Hematology and Medical Oncology, VA New York Harbor Healthcare System, New York, New York 10010-5050, USA.. jhfliess@mail.med.cornell.edu) . Biochemistry, (2000 Jun 13) 39 (23) 6936-43. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Endothelial cell CD39/ecto-ADPase plays a major role in vascular homeostasis. It rapidly metabolizes ADP released from stimulated platelets, thereby preventing further **platelet activation** and recruitment. We recently developed a recombinant, soluble form of human CD39, solCD39, with enzymatic and biological properties identical to CD39. To identify amino acids essential for enzymatic/biological activity, we performed site-directed mutagenesis within the four highly conserved apyrase regions of solCD39. Mutation of glutamate 174 to alanine (E174A) and serine 218 to alanine (S218A) resulted in complete and approximately 90% loss of solCD39 enzymatic activity, respectively. Furthermore, compared to wild-type, S57A exhibited a 2-fold increase in ADPase activity without change in ATPase activity, while the tyrosine 127 to alanine (Y127A) mutant lost 50-60% of both ADPase and ATPase activity. The ADPase activity of wild-type solCD39 and each mutant, except for R135A, was greater with calcium as the required divalent cation than with magnesium, but for ATPase activity generally no such preference was observed. Y127A demonstrated the highest calcium/magnesium ADPase activity ratio, 2.8-fold higher than that of wild-type, even though its enzyme activity was greatly reduced. SolCD39 mutants were further characterized by correlating enzymatic with biological activity in an in vitro platelet aggregation system. Each solCD39 mutant was similar to wild-type in reversing platelet aggregation, except for E174A and S218A. E174A, completely devoid of enzymatic activity, failed to inhibit platelet responsiveness, as anticipated. S218A, with 91% loss of ADPase activity, could still reverse platelet aggregation, albeit much less effectively than wild-type solCD39. Thus, glutamate 174 and serine 218 are essential for both the enzymatic and biological activity of solCD39.

L15 ANSWER 9 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2001:322309 Document No.: PREV200100322309. Identification of functionally important amino acid residues in soluble human CD39: An important thrombo-regulator. Drosopoulos, J. H. F. [Reprint author]; Broekman, M. J.; Islam, N.; Gayle, R. B., III; Maliszewski, C. R.; Marcus, A. J.. VA NY Harbor Healthcare System, Weill Medical College of Cornell Univ., New York, NY, USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 813a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Endothelial cell ecto-ADPase/CD39 plays a major role in maintenance of blood fluidity. It rapidly metabolizes ADP released from activated platelets, thereby preventing further **platelet activation** and recruitment. We developed a recombinant, soluble form of human CD39, solCD39, with enzymatic and biological properties identical to CD39. To identify amino acids essential for enzymatic/biological activity, we performed site-directed mutagenesis within the highly conserved apyrase regions (ACR) of solCD39. Mutations E174A and S218A resulted in complete and apprx90% loss of enzymatic activity, respectively. Compared to wild-type, S57A displayed a 2-fold increase in ADPase activity with no change in ATPase activity, whereas Y127A lost apprx55% of both ADPase and ATPase activity. D213A showed the greatest increase in both ADPase and ATPase activity. D54A and D213A had 1.5-fold higher enzyme activity with ATP than with ADP as substrate. Enzymatic activity of solCD39 mutants correlated strongly with their biological activity in an in vitro platelet aggregation system. In citrated plasma, each mutant resembled wild-type in reversing platelet aggregation, with the exception of D54A, E174A, D213A, and S218A. E174A, devoid of enzyme activity, did not inhibit platelet reactivity. S218A, with 91% loss of ADPase activity, could still reverse platelet aggregation, albeit much less effectively than wild-type. Interestingly, D54A and D213A had decreased ability to reverse platelet aggregation, even through their ADPase and ATPase activities were greater than that of wild-type in enzymatic assays. In addition, their ADPase activity in the

presence of citrated plasma was also decreased, and this was overcome by addition of excess calcium. The citrate in anticoagulated plasma reduced free calcium to a suboptimal level for full enzymatic activity of D54A and D213A, and decreased their ability to inhibit platelet aggregation as effectively as wild-type solCD39. In heparinized plasma, D54A and D213A completely reversed platelet aggregation and their ADPase activities were similar to that observed in enzyme assays. Kinetic analyses revealed a low binding affinity of D54A and D213A for calcium as well as for ADP and ATP. Decreases in binding were compensated for by increases in rate of catalysis. Thus, aspartates 54 and 213 are involved in calcium binding in the catalytic pocket of solCD39. Glutamate 174 and serine 218 are essential for the enzymatic as well as biological activity of the enzyme. Our study defines amino acid residues required for enzyme catalysis and provides specific information concerning the active site of solCD39 - a potential antithrombotic agent.

=> s IL-2 leader
L16 12 IL-2 LEADER

=> s l116 and CD39
L17 0 LL16 AND CD39

=> dup remove l16
PROCESSING COMPLETED FOR L16
L18 5 DUP REMOVE L16 (7 DUPLICATES REMOVED)

=> d l18 1-5 cbib abs

L18 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
1996:449800 Document No. 125:105092 Plasmids suitable for interleukin-2 gene recombinant expression, gene therapy, and safety of intravenous administration of interleukin-2 plasmid DNA in mice for neoplasm treatment. Hobart, Peter M.; Margalith, Michal; Parker, Suezanne E.; Khatibi, Shirin (Vical Incorporated, USA). PCT Int. Appl. WO 9617063 A1 19960606, 86 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US15020 19951128. PRIORITY: US 1994-345913 19941128.
AB The present invention relates to plasmids suitable for IL-2 expression, particularly, human IL-2 expression, and related methods. The plasmid consists essentially of an expression facilitating sequence derived from the immediate-early promoter region of CMV, an expression facilitating sequence derived from the transcriptional termination-polyadenylation signal sequence of the BGH gene, a sequence coding for the eukaryotic expression of an IL-2, possessing a bioactivity of the complete IL-2, operably linked to both of said expression facilitating sequences. The sequence coding for the expression of an IL-2 is a sequence encoding a mature IL-2 and a non-**IL-2 leader** peptide that augments eukaryotic expression compared to a wild-type **IL-2 leader** peptide. Optionally, the plasmid also includes a non-mammalian origin of a replication and a sequence operably encoding a selectable marker.

L18 ANSWER 2 OF 5 MEDLINE on STN DUPLICATE 1
92276826. PubMed ID: 1534340. Purification and characterization of biologically active human recombinant 37 kDa soluble CD23 (sFc epsilon RII) expressed in insect cells. Graber P; Jansen K; Pochon S; Shields J; Aubonney N; Turcatti G; Bonnefoy J Y. (Glaxo Institute for Molecular Biology S.A., Geneva, Switzerland.) Journal of immunological methods, (1992 May 18) 149 (2) 215-26. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB Human recombinant soluble 37 kDa CD23 has been expressed in insect cells and secreted into the culture medium using the **IL-2 leader** sequence. The 37 kDa CD23 was purified 600-fold to homogeneity by monoclonal antibody affinity chromatography and gel

filtration. The pure protein is monomeric, glycosylated, depleted of one N terminal amino acid and contains four disulphide bonds. It degrades into smaller fragments of 33, 29 and 25 kDa if purified in the absence of protease inhibitors. The same pattern of proteolytic fragments is observed when the pure preparation is incubated at room temperature for 3 weeks. Physical characterization of the 37 kDa CD23 by circular dichroism indicates that the protein contains mainly beta sheet and 20% of alpha helical structures. Specific binding of IgE to natural CD23 (low affinity IgE receptor) was inhibited by purified recombinant 37 kDa CD23. Moreover, purified recombinant 37kDa CD23 and interleukin-1 promoted the survival of germinal centre B cells.

L18 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
1988:588798 Document No. 109:188798 Immunoaffinity column purification of recombinant IgE Fc fragment fusion proteins for use as an antiallergy medicine. Ikeyama, Shuichi; Nishimura, Osamu (Takeda Chemical Industries, Ltd., Japan). Eur. Pat. Appl. EP 269455 A2 19880601, 19 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1987-310475 19871127. PRIORITY: JP 1986-281871 19861128; JP 1987-232295 19870918.

AB Purification of recombinant IgE Fc fragment-interleukin-2 (IL-2) signal peptide fusion proteins using immunoaffinity chromatog. Mouse L cells transformed with pTB543, encoding a fusion protein comprising the **IL-2 leader** peptide, the 1st 11 N-terminal amino acids of IL-2, a small linker peptide, and the Fc portion of human IgE, were cultured. The fusion protein was purified from the medium using (NH4)2SO4 precipitation, immunoaffinity chromatog. (with a monoclonal antibody against IgE), and gel filtration chromatog. (Sephacryl S-200). A pure glycosylated protein was obtained.

L18 ANSWER 4 OF 5 MEDLINE on STN DUPLICATE 2
88253447. PubMed ID: 3260137. Secretion of human EGF and IgE in mammalian cells by recombinant DNA techniques; use of a **IL-2 leader** sequence. Sasada R; Marumoto R; Igarashi K. (Central Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan.) Cell structure and function, (1988 Apr) 13 (2) 129-41. Journal code: 7608465. ISSN: 0386-7196. Pub. country: Japan. Language: English.

AB Expression plasmids were constructed containing chemically synthesized human epidermal growth factor (EGF) gene fused in a frame to a leader sequence of human interleukin-2 (IL-2) gene under the control of a viral promoter. COS7 cells transfected with the plasmids synthesized and secreted EGF. Transfection of mouse A9 cells or BALB/3T3 clone A31 cells with the plasmids permitted the isolation of cell lines secreting the product which showed EGF activity. In particular, A31 transformed cells secreting human EGF grew well even in a medium containing a minimal level of serum. Using similar vectors having IgE cDNA (C2-C4) in place of EGF gene, a human IgE Fc fragment was also produced and secreted in mouse cells. These results show that heterologous leader sequences are useful for the expression and secretion of proteins whose genes lack leader sequences.

L18 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
1987:548599 Document No. 107:148599 Expression vector for manufacture of secretory proteins with animal cells. Igarashi, Koichi; Fujii, Tomoko; Sasada, Reiko; Marumoto, Ryuji (Takeda Chemical Industries, Ltd. , Japan). Eur. Pat. Appl. EP 225701 A1 19870616, 49 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1986-308061 19861017. PRIORITY: JP 1985-236189 19851021; JP 1986-182457 19860801.

AB An expression vector containing a promoter and a DNA sequence encoding human interleukin 2 (IL-2) signal peptide (leader sequence) is constructed for cloning and expression of genes for physiol. active peptides such as epithelial cell growth factor (ECGF) in animal cells and facilitating secretion of these products into the culture medium. Expression vector

pTB410 containing **IL-2 leader** sequence, SV40-derived promoter, splicing site, and poly(A) addition site, and a synthetic human ECGF gene was constructed. Its ClaI-HindIII region (upstream of the SV40 promoter) was further replaced with a fragment containing Abelson mouse leukemia virus LTR sequence to obtain expression vector pTB503. Mouse LA9 cells transformed with pTB505 (comparable to pTB503) produced 80 mg ECGF/mL in the culture supernatant vs. <1 for the control.

=> s CD39
L19 1355 CD39

=> s l19 and alanine
L20 18 L19 AND ALANINE

=> dup remove l20
PROCESSING COMPLETED FOR L20
L21 10 DUP REMOVE L20 (8 DUPLICATES REMOVED)

=> d l21 1-10 cbib abs

L21 ANSWER 1 OF 10 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:651736 The Genuine Article (R) Number: 837VB. Site-directed mutagenesis of human soluble calcium-activated nucleotidase 1 (hSCAN-1): Identification of residues essential for enzyme activity and the Ca2+-induced conformational change. Yang M Y; Kirley T L (Reprint). Univ Cincinnati, Dept Pharmacol & Cell Biophys, Coll Med, POB 370575, Cincinnati, OH 45267 USA (Reprint); Univ Cincinnati, Dept Pharmacol & Cell Biophys, Coll Med, Cincinnati, OH 45267 USA. terry.kirley@uc.edu. BIOCHEMISTRY (20 JUL 2004) Vol. 43, No. 28, pp. 9185-9194. ISSN: 0006-2960 . Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Human soluble calcium-activated nucleotidase 1 (hSCAN-1) is the human homologue of soluble apyrases found in blood-sucking insects. This family of nucleotidases is unrelated in sequence to more well-studied nucleotidases, and very little is known about the enzymatic mechanism. By multiple sequence alignment, eight regions that are highly conserved in the hSCAN-1 family were identified and named. To identify amino acids important for catalytic activity and enzyme specificity, seven point mutations were constructed, expressed in bacteria, refolded, purified, and characterized. Substitution of glutamic acid 130 with tyrosine resulted in dramatically increased nucleotidase activities, while mutagenesis of aspartic acid 151 to **alanine** and aspartic acid 84 to **alanine** completely abolished activity. Mutagenesis of arginine 133 and arginine 271 resulted in enzymes with very little nucleotidase activity. Mutagenesis of aspartic acid 175 to **alanine** and glycine 122 to glutamic acid had smaller negative effects on enzyme activities. Previously, our laboratory showed that calcium triggers a conformational change in hSCAN-1 necessary for nucleotidase activity. Here we show that several mutants (D84A, R133A, and D151A) that lost most of their activity were unable to undergo the conformational change induced by Ca2+, as shown by Cibacron blue binding, limited proteolysis, and tryptophan fluorescence. We conclude that aspartic acid residues 84 and 151, as well as arginine residue 133, are essential for the Ca2+-induced conformational change that is necessary for enzyme activity. Aspartic acid 175 and glutamic acid 130 are important for determining substrate specificity. In addition, we show that Sr2+, unlike Mg2+ and other divalent cations, can substitute for Ca2+ to induce the conformational change necessary for enzyme activity. However, Sr2+ cannot substitute for Ca2+ to support nucleotide hydrolysis, presumably because Sr2+ cannot substitute for Ca2+ in its second role as a nucleotide cosubstrate. The ramifications of our results on the interpretation of a recently published

crystal structure are discussed. This information will facilitate future engineering of this enzyme designed to enhance its ability to hydrolyze ADP and thus increase its potential for therapeutic use in the treatment of pathological ischemic events triggered via activation of platelets by ADP.

L21 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2002:11124 Document No. 136:79766 Inhibitors of platelet activation and recruitment. Maliszewski, Charles Richard; Gayle, Richard Brownley; Price, Virginia Lee; Gimpel, Steven Dean (USA). U.S. Pat. Appl. Publ. US 2002002277 A1 20020103, 78 pp., Cont.-in-part of Appl. No. PCT/US99/22955. (English). CODEN: USXXCO. APPLICATION: US 2001-835147 20010413. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813; WO 1999-US22955 19991013.
AB The present invention provides soluble **CD39** polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble **CD39** polypeptide.

L21 ANSWER 3 OF 10 MEDLINE on STN DUPLICATE 1
2002472791. PubMed ID: 12234494. Roles of Asp54 and Asp213 in Ca2+ utilization by soluble human **CD39**/ecto-nucleotidase. Drosopoulos Joan H F. (Research Service, Thrombosis Research Laboratory, VA New York Harbor Healthcare System, New York, NY 10010-5050, USA.. jhfliess@med.cornell.edu) . Archives of biochemistry and biophysics, (2002 Oct 1) 406 (1) 85-95. Journal code: 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.
AB Soluble human **CD39** (solCD39) rapidly metabolizes nucleotides, especially ADP released from activated platelets, thereby inhibiting further platelet activation and recruitment. Using **alanine** substitution mutagenesis, we established a functional role for aspartates D54 and D213 in solCD39. Kinetic analyses of D54A and D213A indicated decreased K(m)s of the mutants, compared to wild type, for the cofactor calcium and for the substrates ADP and ATP. These decreases in calcium and nucleotide affinity of the mutants were accompanied by increases in their rate of catalysis. The decreased affinity of the mutants for calcium was responsible for their diminished ability to reverse platelet aggregation in plasma anticoagulated with citrate, a known calcium chelator. Their ADPase activity in the presence of citrated plasma was also decreased, although this could be overcome with excess calcium. Thus, aspartates 54 and 213 are involved in calcium utilization and potentially involved in cation coordination with substrate in the catalytic pocket of solCD39.

L21 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2001:828425 Document No. 137:89413 Detection of variations in the DNA methylation profile of genes in the determining the risk of disease. Berlin, Kurt; Piepenbrock, Christian; Olek, Alexander (Epigenomics A.-G., Germany). PCT Int. Appl. WO 2001077373 A2 20011018, 636 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (German). CODEN: PIXXD2. APPLICATION: WO 2001-XB1486 20010406. PRIORITY: DE 2000-10019058 20000406; WO 2001-DE1486 20010406.
AB The invention relates to an oligonucleotide kit as probe for the detection of relevant variations in the DNA methylation of a target group of genes. The invention further relates to the use of the same for determining the gene variant with regard to DNA methylation, a medical device, using an oligonucleotide kit, a method for determining the methylation state of an individual and a method for the establishment of a model for establishing the probability of onset of a disease state in an individual. Such diseases may be: undesired pharmaceutical side-effects; cancerous

diseases; CNS dysfunctions, injuries or diseases; aggressive symptoms or relational disturbances; clin., psychol. and social consequences of brain injury; psychotic disorders and personality disorders; dementia and/or associated syndromes; cardiovascular disease, dysfunction and damage; dysfunction, damage or disease of the gastrointestinal tract; dysfunction, damage or disease of the respiratory system; injury, inflammation, infection, immunity and/or anastasis; dysfunction, damage or disease of the body as an abnormal development process; dysfunction, damage or disease of the skin, muscle, connective tissue or bones; endocrine and metabolic dysfunction, damage or disease; headaches or sexual dysfunction. This abstract record is one of several records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.

L21 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

2001:114897 Document No. 134:174556 Human **CD39**-like proteins and cDNAs and methods for drug screening and antithrombosis therapy. Ford, John; Mulero, Julio J.; Yeung, George (Hyseq Inc., USA). PCT Int. Appl. WO 2001010205 A1 20010215, 203 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US21790 20000809. PRIORITY: US 1999-370265 19990809; US 2000-481238 20000111; US 2000-557800 20000425; US 2000-583231 20000526; US 2000-608285 20000630.

AB The invention provides human **CD39**-like proteins and their cDNAs. The **CD39**-L2, **CD39**-L4, and **CD39**-L66 proteins possess apyrase activity. Other aspects of the invention include vectors containing DNA of the invention, recombinant host cells expressing the DNA, processes for producing the **CD39**-like proteins, and antibodies specific for the proteins. Also disclosed are use of the **CD39**-like proteins as antithrombotics and for screening for modulators of the function of the **CD39**-like proteins. Thus, the cDNAs for human apyrases designated **CD39**-L2, **CD39**-L4, and **CD39**-L66 were cloned, sequenced, and expressed in COS7, 293 and insect cells, and their apyrase activity demonstrated. These appear to be a new class of E-type apyrase with a specificity for NDPs as substrates. Site-specific mutants of **CD39**-L4 were prepared with enhanced apyrase activity. The human and mouse genes were also cloned and the gene for **CD39**-L4 was mapped to human chromosome 11.

L21 ANSWER 6 OF 10 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:294426 The Genuine Article (R) Number: 416UM. Site-directed mutagenesis of human nucleoside triphosphate diphosphohydrolase 3: The importance of residues in the apyrase conserved regions. Yang F; Hicks-Berger C A; Smith T M; Kirley T L (Reprint). Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, 231 Bethesda Ave, POB 670575, Cincinnati, OH 45267 USA (Reprint); Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, Cincinnati, OH 45267 USA; Armstrong Atlantic State Univ, Dept Biol, Savannah, GA 31419 USA. BIOCHEMISTRY (3 APR 2001) Vol. 40, No. 13, pp. 3943-3950. ISSN: 0006-2960. Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Ecto-nucleoside triphosphate diphosphohydrolase 3 (eNTPDase-3, also known as HB6 and CD39L3) is a membrane-associated ecto-apyrase. Only a few functionally significant residues have been elucidated for this enzyme, as well as for the whole family of eNTPDase enzymes. Four highly conserved regions (apyrase conserved regions, ACRs) have been identified in all the members of eNTPDase family, suggesting their importance for biological activity. In an effort to identify those amino acids important

for the catalytic activity of the eNTPDase family, as well as those residues mediating substrate specificity, 11 point mutations of 7 amino acid residues in ACR1-4 of eNTPDase-3 were constructed by site-directed mutagenesis. Mutagenesis of asparagine 191 to **alanine** (N191A), glutamine 226 to **alanine** (Q226A), and arginine 67 to glycine (R67G) resulted in an increase in the rates of hydrolysis of nucleoside diphosphates relative to triphosphates. Mutagenesis of arginine 146 to proline (R146P) essentially converted the eNTPDase-3 ecto-apyrase to an ecto-ATPase (eNTPDase-2), mainly by decreasing the hydrolysis rates for nucleoside diphosphates. The Q226A mutant exhibited a change in the divalent cation requirement for nucleotidase activity relative to the wild-type and the other mutants. Mutation of glutamate 182 to aspartate (E182D) or glutamine (E182Q), and mutation of serine 224 to **alanine** (S224A) completely abolished enzymatic activity. We conclude that the residues corresponding to eNTPDase-3 glutamate 182 in ACR3 and serine 224 in ACR4 are essential for the enzymatic activity of eNTPDases in general, and that arginine 67, arginine 146, asparagine 191, and glutamine 226 are important for determining substrate specificity for human ecto-nucleoside triphosphate diphosphohydrolase 3.

L21 ANSWER 7 OF 10 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:911174 The Genuine Article (R) Number: 493TM. Site-directed mutagenesis of human nucleoside triphosphate diphosphohydrolase 3: The importance of conserved glycine residues and the identification of additional conserved protein motifs in eNTPDases. Kirley T L (Reprint); Yang F; Ivanenkov V V. Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, 231 Albert Sabin Way, POB 670575, Cincinnati, OH 45267 USA (Reprint); Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, Cincinnati, OH 45267 USA. ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS (1 NOV 2001) Vol. 395, No. 1, pp. 94-102. ISSN: 0003-9861. Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Glycine residues are recognized as important structural determinants in nucleotide-binding domains of many enzymes. The functional significance of seven glycine residues invariant in all 22 eNTPDase sequences was therefore examined. Glycine-to-**alanine** mutants of eNTPDase3 were analyzed for nucleotidase activities and tertiary and quaternary structure changes. Mutations G98A and G183A had modest effects on ATPase and ADPase activities. The G141A mutation resulted in 4- to 5-fold decreased nucleotidase activity, while the G222A mutation decreased ATPase activity 20-fold, and ADPase activity 6-fold. Unlike the other five glycine mutants, the G263A and G462A mutations caused significant loss of nucleotidase activity which was observed concomitant with lower protein expression levels, large-scale changes in tertiary and quaternary protein structure, and decreased trafficking to the plasma membrane. Thus, these data identify glycine residues that are essential for enzymatic activity and the tertiary and quaternary structure of eNTPDase3. Further, two additional conserved regions in the eNTPDases are identified, apyrase conserved regions ACR1a and ACR4a, which may be involved in phosphate binding/hydrolysis and protein folding, respectively. (C) 2001 Academic Press.

L21 ANSWER 8 OF 10 MEDLINE on STN DUPLICATE 2

2001241856. PubMed ID: 11343793. The importance of histidine residues in human ecto-nucleoside triphosphate diphosphohydrolase-3 as determined by site-directed mutagenesis. Hicks-Berger C A; Yang F; Smith T M; Kirley T L. (Department of Pharmacology and Cell Biophysics, College of Medicine, University of Cincinnati, OH 45267-0575, USA.) Biochimica et biophysica acta, (2001 May 5) 1547 (1) 72-81. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Most ecto-nucleoside triphosphate diphosphohydrolases (eNTPDases) are inhibited by the histidine reagent diethyl pyrocarbonate (DEPC), while being resistant to inhibition by many other chemical modification agents. We used site-directed mutagenesis to investigate the sites of modification

responsible for DEPC inhibition. First, we constructed the mutations H135A and R67H in eNTPDase-3 to address the possibility that, in eNTPDase-3, histidine 135 compensates for the lack of a histidine in apyrase conserved region (ACR) 1, present in all other membranous eNTPDases (but replaced by R67 in ACR1 of eNTPDase-3). We found histidine 135 is a major, but not the sole, target for DEPC-induced inhibition in eNTPDase-3. In addition, analysis of the R67H mutant led us to conclude that this site is important for DEPC inactivation of other eNTPDases. We also mutated singly and collectively three of the most conserved histidine residues present in eNTPDase-3 (129, 257 and 447) to **alanine.** None of the single, conserved histidine mutations nor the triple histidine mutation inactivated the enzyme or decreased susceptibility to DEPC inhibition. However, changes in the tendency of monomers to self-associate were noted, and the triple histidine mutant exhibited a higher nucleotidase specific activity than the wild-type.

L21 ANSWER 9 OF 10 MEDLINE on STN DUPLICATE 3

2000302517. PubMed ID: 10841775. Site-directed mutagenesis of human endothelial cell ecto-ADPase/soluble **CD39**: requirement of glutamate 174 and serine 218 for enzyme activity and inhibition of platelet recruitment. Drosopoulos J H; Broekman M J; Islam N; Maliszewski C R; Gayle R B 3rd; Marcus A J. (Department of Medicine, Division of Hematology and Medical Oncology, VA New York Harbor Healthcare System, New York, New York 10010-5050, USA.. jhfliess@mail.med.cornell.edu) . Biochemistry, (2000 Jun 13) 39 (23) 6936-43. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Endothelial cell **CD39**/ecto-ADPase plays a major role in vascular homeostasis. It rapidly metabolizes ADP released from stimulated platelets, thereby preventing further platelet activation and recruitment. We recently developed a recombinant, soluble form of human **CD39**, solCD39, with enzymatic and biological properties identical to **CD39.** To identify amino acids essential for enzymatic/biological activity, we performed site-directed mutagenesis within the four highly conserved apyrase regions of solCD39. Mutation of glutamate 174 to **alanine** (E174A) and serine 218 to **alanine** (S218A) resulted in complete and approximately 90% loss of solCD39 enzymatic activity, respectively. Furthermore, compared to wild-type, S57A exhibited a 2-fold increase in ADPase activity without change in ATPase activity, while the tyrosine 127 to **alanine** (Y127A) mutant lost 50-60% of both ADPase and ATPase activity. The ADPase activity of wild-type solCD39 and each mutant, except for R135A, was greater with calcium as the required divalent cation than with magnesium, but for ATPase activity generally no such preference was observed. Y127A demonstrated the highest calcium/magnesium ADPase activity ratio, 2.8-fold higher than that of wild-type, even though its enzyme activity was greatly reduced. SolCD39 mutants were further characterized by correlating enzymatic with biological activity in an in vitro platelet aggregation system. Each solCD39 mutant was similar to wild-type in reversing platelet aggregation, except for E174A and S218A. E174A, completely devoid of enzymatic activity, failed to inhibit platelet responsiveness, as anticipated. S218A, with 91% loss of ADPase activity, could still reverse platelet aggregation, albeit much less effectively than wild-type solCD39. Thus, glutamate 174 and serine 218 are essential for both the enzymatic and biological activity of solCD39.

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1999:343714 The Genuine Article (R) Number: 193YP. Mutagenesis of two conserved tryptophan residues of the E-type ATPases: Inactivation and conversion of an ecto-apyrase to an Ecto-NTPase. Smith T M; Carl S A L; Kirley T L (Reprint). Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, 231 Bethesda Ave, POB 670575, Cincinnati, OH 45267 USA (Reprint); Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, Cincinnati, OH 45267 USA. BIOCHEMISTRY (4 MAY 1999) Vol. 38, No. 18, pp. 5849-5857. ISSN: 0006-2960. Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC

20036 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A human brain E-type ATPase (HB6 ecto-apyrase) was subjected to site-directed mutagenesis to assess the functional significance of two highly conserved tryptophan residues (Trp 187 and Trp 459), the only two tryptophans conserved in nearly all E-type ATPases. Mutation of tryptophan 187 to **alanine** yielded a poorly expressed ecto-apyrase completely devoid of nucleotidase activity. Immunolocalization of the W187A mutant in mammalian COS cells showed a cellular distribution clearly different from that of the wild-type enzyme, with the majority of the immunoreactivity concentrated in the interior of the cell. Unlike the wild-type enzyme, this mutant did not bind the nucleotide analogue Cibacron Blue and was sensitive to proteolytic digestion by chymotrypsin. These results suggest alteration of the tertiary structure, causing the enzyme to be improperly folded and retained within the cell. In contrast, mutation of tryptophan 459 to **alanine** resulted in an ecto-apyrase with enhanced NTPase-activity, but diminished NDPase activity. Immunolocalization of this active mutant ecto-apyrase revealed a cellular pattern similar to that of the wild-type enzyme, distributed along the cell periphery and in cell processes. Coupling this active W459A mutation to a previously described mutation (D219E) resulted in an enzyme which preferentially hydrolyzes nucleoside triphosphates over diphosphates. The D219E/W459A double mutant had an ATPase:ADPase ratio of 11:1 and a UTPase:UDPase ratio of 148:1. In addition, the double mutant is substantially less sensitive to inhibition by azide, a more potent inhibitor of ecto-apyrases than ecto-ATPases. Thus, mutation of only two amino acids of an E-type ATPase essentially converts an ecto-apyrase to an ecto-NTPase.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	322.26	322.47

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-26.28	-26.28

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1: Cell Struct Funct. 1988 Apr;13(2):129-41.

Secretion of human EGF and IgE in mammalian cells by recombinant DNA techniques; use of a IL-2 leader sequence.

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Central Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan.

Expression plasmids were constructed containing chemically synthesized human epidermal growth factor (EGF) gene fused in a frame to a leader sequence of human interleukin-2 (IL-2) gene under the control of a viral promoter. COS7 cells transfected with the plasmids synthesized and secreted EGF. Transfection of mouse A9 cells or BALB/3T3 clone A31 cells with the plasmids permitted the isolation of cell lines secreting the product which showed EGF activity. In particular, A31 transformed cells secreting human EGF grew well even in a medium containing a minimal level of serum. Using similar vectors having IgE cDNA (C2-C4) in place of EGF gene, a human IgE Fc fragment was also produced and secreted in mouse cells. These results show that heterologous leader sequences are useful for the expression and secretion of proteins whose genes lack leader sequences.

PMID: 3260137 [PubMed - indexed for MEDLINE]